Protein Related Products

MANUAL GUIDE

PART VII
in vitro Transcription & Translation Kit

Products	Cat. No.	Size
Genelator™ *in vitro* Transcription/Translation Mini Kit	12011	25 ea. x 45μℓ rxn.
Genelator™ *in vitro* Transcription/Translation Midi Kit	12012	10 ea. x 900μℓ rxn.
F-Detector™ Cell Free Labeling Mini Kit	12021	60 μℓ
F-Detector™ Cell Free Labeling Midi Kit	12022	300 μℓ

iNtRON BIOTECHNOLOGY

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강한 대한민국!

Power Korea
That's it

**제품은 만드는 것만이 중요한 것이 아닙니다.
완벽한 품질관리를 통한 일정한 품질유지가
비로소 제품을 완성시킵니다.**

국립수의과학검역원, 식품의약품안전청, 국립보건원, 질병관리통제센터, 농업진흥청, 농업생명공학원 축산기술원, 한국담배인삼공사 중앙연구소, 호남농업시험장, 영남농업시험장, 서울대학교, 연세대학교, 고려대학교, 성균관대학교, 경희대학교, 한양대학교, 건국대학교, 이화여대, 숙명여대, 세종대학교, 상명대학교, 충북대학교, 충남대학교, 전북대학교, 전남대학교, 목원대학교, 원광대학교, 중앙대학교, 명지대학교, 동국대학교, 상명대학교, 세종대학교, 강원대학교, 국민대학교, 인하대학교, 경상대학교, 배재대학교, 서울대병원, 연세의료원, 고려대병원, 삼성서울병원, 아산병원, KIST, 삼성제일병원, 강남성모병원, 여의도성모병원, 이대병원, 원자력병원, 기초과학지원센터, 두산기술원, 마크로젠, 조선대학교, 순천향 대학교, 광주과학기술원, 강릉대학교, 제일바이오, 영남대학교, 진주산업대학교, 인제대학교, 한국야쿠르트, 포항공과대학교, 영남대학교, 경북대학교, 씨제이, 아주대학교, 아주의료원, 가천의과대학교, 농우바이오, 한경대학교, 굿젠, 포천중문의과대학교, 바이오세움, 한국야쿠르트, 동아제약, 태평양, 녹십자, 원예연구소, 한국외국어대학교, 부산대학교, 풀무원, 종근당, 단국대학교, 울산대학교, 경희의료원, 카톨릭대학교, 건양대학교, 대전대학교, 청주대학교, 공주대학교, 제주대학교, 전주대학교, 을지병원, 엘지생명과학기술연구원, 대상사료주식회사, 원예연구소, 작물시험장, 국립종자관리소, 분당차병원. 인제대학, 국립암센터, 에스케이제약, 생명공학연구원, 과학기술원, 화학연구원, 농업기술원, 결핵연구원, 소비자보호원, 녹십자목암연구소, 영동세브란스병원 外 전국 정부기관연구소 및 대학연구소, 그리고 유수의 대기업 및 중소/벤처/기업연구소, Dartmouth college Department Microbiology, University of Washington, Institute of Molecular Medicine Trinity Center for Health Sciences St. James Hospital, Johns Hopkins Applied Physics Laboratory, University of Texas at Austin Dept. of Molecular Genetics & Microbiology University Station Baylor College of Medicine, University of Perdue, EMBL (European Molecular Biology Laboratory), University of Washington & Harborview Medical Center, Università degli Studi di Torino, Calbiochem Ltd, School of Marine Science Virginia Institute of Marine Science College of William and Mary, Center for Experimental Pathology Cantonal Institute of Pathology, R&D Immunology OXOID Ltd., ID Biomedical Corps of Quebec, Intervet Ltd, Iantechno srl Italy, Fachhochschule Aachen Germany, Infectious research center Laval University Hospital Center, School of Dentistry (Oral Biology) The University of Birmingham, Panum Institute Copenhagen, Sertoli Cell Group University of Glasgow 등, 많은 분들이 ㈜인트론바이오테크놀로지의 우수한 제품을 사용하고 계십니다. 감사합니다.

**대한민국을 빛내고 계시는 우수한 여러분들이
나라의 이름을 떠받치며 경쟁력을 쌓고 계시고,
바로 이러한 노력에 미약하나마 힘이 되리라는 최선의 의지로
저희 인트론은 사명감을 가지고 제품을 만들고 공급하고 있습니다.
우리는 이미 세계로 나아가고 있습니다.
이미 많은 분들이 미래를 만들고 계십니다.**

※ 상기 리스트는 당사 제품 공급자료를 기초로 나열된 것이며, 특별한 순서는 없습니다.

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여러분의 실험실에 "혁명"이 일어납니다.

뜯어서 바로 쓰는
바로젤 !!

PRE-made Agarose Gel for TBE buffer (1.5%)
Cat.No. 21051 (32 packs/Box)

Gel 판과 Gel Cast, Agarose Gel이 모두 한 Pack에 들어 있습니다.
여러분은 포장을 뜯어서 전기영동장치에 꽂아만 주십시오.
※ 17 well 이 기본으로 제공됩니다.

아끼십시오 !!!
Gel 만드는데 걸리는 시간 = 2시간
만드는 과정에서 EtBr에 노출되는 여러분의 건강

"바로젤"을
사용하시는 순간,
여러분은
행복해집니다.

실험실에는
하고 싶은 일과,
하기 싫은 일이
있습니다...

저희 인트론은
소중한 여러분이
하고 싶은 일만
하실 수 있도록
최선을 다합니다.

바로 지금,
인트론을
찾으세요 !!

제품주문

전 화 : 0505-550-5600
팩 스 : 0505-550-5660
온라인 : www.intronbio.com
이메일 :info@intronbio.com

ORDER INFORMATION

Please include the following information when placing your order.

1) Institution or customer account number
2) Shipping address
3) Billing address
4) Purchase order number
5) Name of end user
6) Telephone of end user
7) Name of purchasing agent
8) Quantity, catalog number, and the description of the product
9) For Visa or MasterCard orders, please provide card type, name on the card, account number, and expiration date

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Order by FAX (+82)505-550-5660, WR or 031-736-7245, KR
Order by E-mail intronbio@intronbio.com info@intronbio.com

PRODUCT INFORMATION

✍ PRODUCT LIMITATION

All iNtRON products are "For research use only and are not intended for diagnostic, therapeutic or any *in vivo* use in Human subjects".

✍ TECHNICAL SERVICES

iNtRON offers a full range of technical services that include a wide range of analytical capabilities and services. In addition to our analytical services, we offer application testing for different molecular biology, cell culture, clinical, and diagnostic reagents.

✍ QUALITY GUARANTEE

iNtRON provides the scientific community with professional-quality laboratory products. If you are not satisfied with a iNtRON product, write to iNtRON Customer Service Department, 358-11, Sangdaewon-Dong, Joongwon-Ku, Kyungki-Do, 462-120, KOREA.

✍ CUSTOM SERVICES

If you have any questions for our products, please contact us.

TEL (+82)505-550-5600, WR or 031-730-7270, KR
E-mail intronbio@intronbio.com

CONTENTS

Product Information

Genelator™ in vitro Transcription/Translation Kit

Mini Kit CAT. NO. 12011	Midi Kit CAT. NO. 12012
25 ea. X 45 μℓ reaction (1.125ml reaction volume) (1) Genelator™ Master Mix 25 tubes containing 33.6 μℓ (2) RNase-Free dH_2O 1 ml (3) Positive-Control DNA (EGFP vector) 200ng/μℓ x 20 μℓ (4) Handbook Guide	10 ea. X 900μℓ reaction (9ml reaction volume) (1) Genelator™ Master Mix 10 tubes containing 672μℓ (2) RNase-Free dH_2O 1 ml x 2 ea. (3) Positive-Control DNA (EGFP vector) 200ng/μℓ x 20μℓ x 2 ea. (4) Handbook Guide

F-Detector™ Cell Free Labeling Kit

CAT. NO. 12021	CAT. NO. 12022
Total 60μℓ (<3μℓ per Genelator™ 45μℓ rxn.) (1) F-Detector™ Fluorescent tRNA one tube containing 60 μℓ mixture (2) Handbook Guide	Total 300μℓ (<3μℓ per Genelator™ 45μℓ rxn.) (1) F-Detector™ Fluorescent tRNA 5 tubes containing 60μℓ mixture (2) Handbook Guide

Intellectual Properties

These **Genelator™** and **F-Detector™** are co-developed with DreamBiogen, Co., Ltd. (www.dreambiogen.com). Furthermore, Materials in this application and Methods of use are covered by various Intellectual Properties.

Trademarks

Genelator™, F-Detector™, DNA-spin™, MEGA-spin™, PCRquick-spin™ (iNtRON)

Patent & Patent Pending

KR10-24695	KR10-131166	KR10-401296	KR10-399337
US5,593,856	KR10-009628	PCT/KR2004/000302	
JP2001-47494	JP2001-57514	US09/783,721	US09/792,647
EP01420032.3	EP01420050.0		

Notice Before Use

Storage and Stability

The **Genelator™ *in vitro* Transcription/Translation Kit** is shipped on dry ice. All components must be stored at –70 ℃ or –20 ℃ . Once thawed, Genelator™ Master Mix should be stored on ice and used within 4 hours. Do not refreeze and thaw more than five times. These temperature fluctuations can greatly alter product stability. When stored under the above conditions and handled correctly, both kits can be kept for at least 6 months without showing any reduction in performance.

Product Use Limitations

The Genelator™ Kit is developed, designed, and sold for research purposes only. It is not to be used for human diagnostic or drug purposes or to be administered to humans. All due care and attention should be exercised in the handling of many of the materials described in this text.

Technical Assistance

At iNtRON we pride ourselves on the quality and availability of our technical support. Our Technical Service Departments are staffed by experienced scientists with extensive practical and theoretical expertise in molecular biology and the use of iNtRON products. If you have any questions or experience any difficulties regarding the Genelator™ and F-Detector™ Kit or iNtRON products in general, please do not hesitate to contact us.

Product Warranty and Satisfaction Guarantee

iNtRON guarantees the performance of all products in the manner described in our product literature. The customer must determine the suitability of the product for its particular use. Should any product fail to perform satisfactorily due to any reason other than misuse, iNtRON will replace it free of charge. We reserve the right to change, alter, or modify any product to enhance its performance and design. If a iNtRON product does not meet your expectations, simply call us.

Safety Information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles.

If any problems in using our products, please call us.

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Product Background

Cell-Free Protein Synthesis (*in vitro* Transcription/Translation)

The Human Genome Project has led to a greater understanding of human genes. Following complete sequencing of the human genome, we are to about to enter a post-genome era in which the function and structure of proteins encoded by the sequenced genes need to be studied. Therefore, great efforts have been made to develop technologies for the production of proteins using recombinant technology. Using modern protein engineering methods, which include cloning of DNA sequences and the *in vivo* expression of genes, it is possible to produce specific proteins in large amounts and also to produce proteins with improved or altered biological activities.

Several factors must be carefully considered when producing recombinant proteins using *in vivo* expression methods. Cells must be transformed with an expression construct (plasmid/vector DNA), and transformants containing the correct construct must be selected and cultivated. However, the **Cell-Based Direct Protein Expression** method has some problems. Firstly, the protein to be used in analysis should be produced in a soluble form. The most common technique for Cell-Based Direct Protein Expression involves using a microorganism such as *E. coli*. The majority of proteins expressed by this *in vivo* expression method are, however, produced in an insoluble or aggregate form such as inclusion bodies.

Although solubilization/refolding procedures are known to transform these proteins into soluble proteins, these are very time-consuming and often inefficient processes, and also they are not generally applicable to all proteins. It is thus most desirable to obtain soluble proteins directly without the requirement for refolding. The production of inactive, insoluble proteins during heterologous cell-based expression results from the fact that, owing to the difference between the rate of protein synthesis and the rate of protein folding, interactions occur between hydrophobic residues exposed from the folding intermediate of the protein of interest, thereby aggregates are formed.

In order to obtain soluble proteins in cell-based expression systems, many methods are utilized, for example, protein engineering approaches such as substitution of original amino acids with a different amino acids to improve solubility of proteins; fermentational approaches such as temperature adjustment, pH adjustment and/or addition of additives; fusing approaches whereby the protein of interest is fused to proteins of high solubility; and co-expression approaches whereby foldases such as DsbA or PPIase are simultaneously expressed with the desired protein. In addition, there is another technique involving co-expression with a chaperone family protein such as GroEL/GroES or DnaK/DnaJ/GrpE for the production of the soluble protein of interest. Molecular chaperones used in Genelator™ System are a set of proteins which participate in protein folding and prevent the aggregation of newly synthesized proteins and lead to the correctly folded protein.

In Cell-Based Direct Protein Expression, overexpression of proteins that are toxic to the host cells can be difficult. Cell lysis and procedures used for purification of protein from whole cell lysates can be complicated; problems may arise because of aggregation or degradation of proteins within the cell. In most cases these limitations can be overcome by the use of **Cell-Free Protein Synthesis Systems** which are a very attractive alternative to classical *in vivo* expression systems.

Cell-Free Protein Synthesis (also termed *in vitro* transcription/translation system) has been a standard tool for the rapid characterization of gene products of interest for almost two decades. The use of cell-free systems for the *in vitro* expression of proteins continues to rapidly expand with various applications in basic research, molecular diagnostics and high-throughput target/drug discovery. Increasingly, extract-based expression tools are being used in functional genomic applications as a "bridge" between traditional genomic and proteomic approaches. Research is moving toward the systematic characterization of gene expression and protein function. *In vitro* expression technologies offer significant time-savings over cellular and whole animal approaches and are generally easy to perform. High-throughput protein-expression screening based on cell-free protein synthesis is useful for large-scale proteomic efforts aimed at identifying and characterizing expressed proteins.

In vitro transcription and translation generates proteins by coupled or successive transcription and translation in cell-free extracts of prokaryotic or eukaryotic cells. The advantages of *in vitro* transcription/translation systems include significant time savings, the possibility to produce proteins that are toxic or contain modified or isotope-labeled amino acids, a high protein yield per unit volume, and the ability to adapt reaction conditions to the requirements of the synthesized protein (e.g., the inclusion of protease inhibitors). Proteins produced by *in vitro* trasnscription/translation can be used for a wide variety of downstream applications, including activity assays, structural and mutational analyses, protein–protein interaction studies, and the expression and analysis of open-reading frames. iNtRON

Genelator™ Information

Gene Translator

Genelator™ *in vitro* Transcription/Translation Mini/Midi Kit

Description

Many proteins are inherently poorly expressed, insoluble, cytotoxic, or susceptible to proteolysis, any of which can result in low-soluble *in vivo* expression yield. Although solubilization/refolding procedures are known to transform these proteins into soluble proteins, these are very time-consuming and often inefficient processes, and also they are not generally applicable to all proteins. It is thus most desirable to obtain soluble proteins directly without the requirement for refolding. For this requirement, **Cell-Free Protein Synthesis** can be considered as an alternative due to its flexibility in manipulating protein folding.

In spite of such an advantage in promoting protein folding, production of aggregation-prone proteins originated from eukaryote using conventional prokaryotic cell-free protein synthesis systems based on *E. coli* frequently results in rapid aggregation of these proteins, although to a lesser extent than in cell-based *in vivo* expression systems. This aggregation is problematic for direct functional analysis using cell-free translation mixture without purification.

The **Genelator™ *in vitro* Transcription/Translation Kit** uses highly productive *E. coli* lysates, which contain all transcriptional and translational machinery components (chaperones, ribosomes, ribosomal factors, tRNAs, aminoacyl-tRNA synthetases, etc.) as well as T7 RNA polymerase. It is a coupled transcription–translation system that can be used to express full-length proteins from T7 or *E. coli* promoters in a single-step reaction using supercoiled (plasmid) DNA templates. Using the **Genelator™** Kit, up to 600 μg/ml biologically active protein can be synthesized within 60 minutes. The synthesis reaction can be easily scaled up (to produce mg amounts of protein). The amount of protein synthesized increases linearly with increased reaction volume.

The **Genelator™ *in vitro* Transcription/Translation Kit** is more suitable for the enhanced soluble production of aggregation-prone protein compared to conventional cell-free protein synthesis. **Genelator™** Kit is prepared from genetically engineered *E. coli* strains which are modified to have enhanced levels of folding-related factors as a folding aid for better solubility of proteins. **Genelator™** Kit serves as a powerful technology for the production of aggregation-sensitive proteins originated from eukaryotes.

We expect this **Genelator™** Kit will be helpful in production a higher level of soluble proteins for further biochemical analysis and proteomics research such as structural genomics and functional genomics. And it is applicable to preparation of protein samples required in the actively growing research areas such as structural genomics, functional genomics, protein chip, diagnosis, protein engineering, bioelectronics including biosensor, and lab-on-a-chip. iNtRON

Genelator™ *in vitro* Transcription/Translation Kit

Mini Kit CAT. NO. 12011	Midi Kit CAT. NO. 12012
25 ea. X 45 μℓ reaction (1.125ml reaction volume) (1) Genelator™ Master Mix 25 tubes containing 33.6 μℓ (2) RNase-Free dH_2O 1 ml (3) Positive-Control DNA (EGFP vector) 200ng/μℓ x 20 μℓ (4) Handbook Guide	10 ea. X 900μℓ reaction (9ml reaction volume) (1) Genelator™ Master Mix 10 tubes containing 672μℓ (2) RNase-Free dH_2O 1 ml x 2 ea. (3) Positive-Control DNA (EGFP vector) 200ng/μℓ x 20μℓ x 2 ea. (4) Handbook Guide

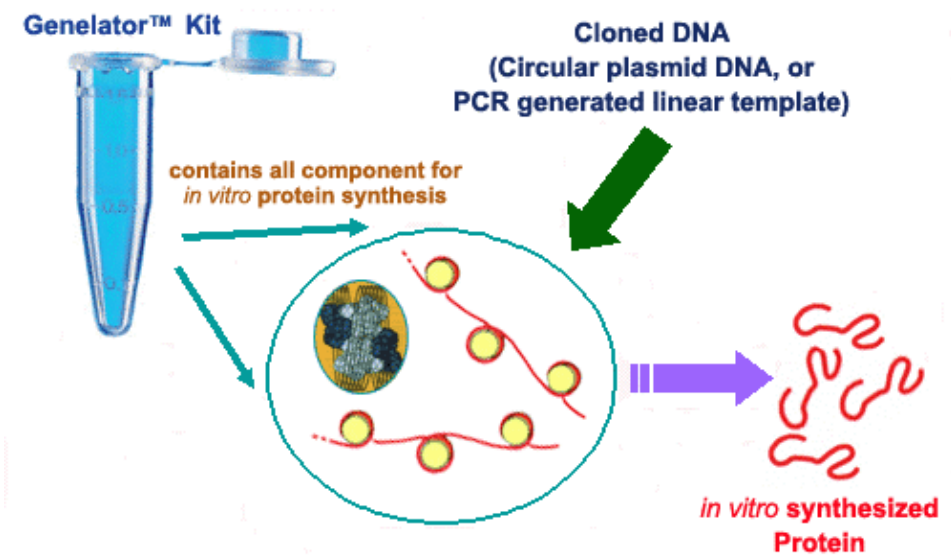
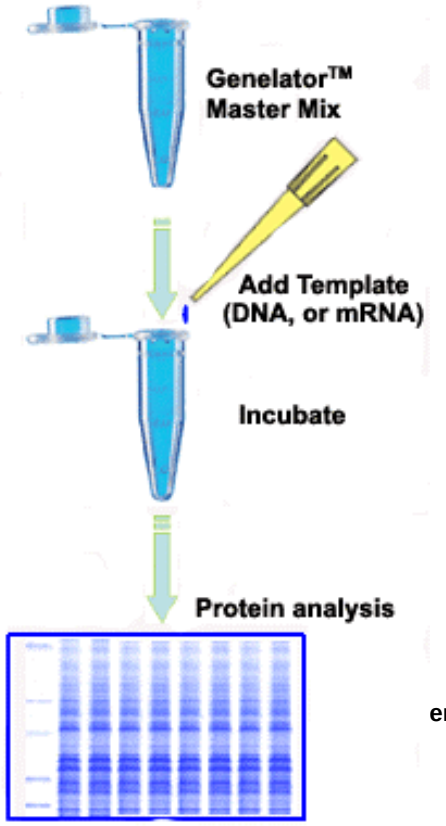


Fig 1. Schematic Showing *in vitro* Transcription/Translation of Proteins

Genelator™ *in vitro* Transcription/Translation Mini/Midi Kit



ematic Drawing of Genelator™ Kit

Very Simple
Very Convenient
Very Fast

High Yield of
Soluble protein

Plasmid DNA Templates

The **Genelator™** Kit can be used to express proteins from a variety of DNA templates as long as they contain a T7 or strong *E. coli* promoter such as Lac promoter. Suitable DNA templates include supercoiled plasmids. The greatest yields of protein are obtained using template DNA of the highest purity. We recommend our **DNA-spin™ Plasmid DNA Purification Kit (CAT. 17093)**. High purity plasmid DNA need to suitable for *in vitro* transcription/translation system. And more, DNA must be free of RNases. The amount of plasmid DNA added to each 45 μℓ *in vitro* transcription and translation reaction should be 0.5 μg for plasmids up to 5 kb in size, or 1 μg for plasmids >5 kb.

Do Not Use RNase

This *in vitro* transcription/translation kit is RNA handling experiment. This system is extremely sensitive to RNase. You should be careful to use this kit for not contamination of RNase.

Purification of *in vitro* Synthesized Proteins

The protein synthesized by **Genelator™** Kit can be easily purified using the conventional following methods.

1) Purification of tagged proteins using the His-tag–Ni-NTA interaction
2) Additional purification using the *Streptavidin* method

Please contact us if you have any questions for purification of proteins.

Notes For Preparing Plasmid DNA

The high purity of plasmid DNA is very important for *in vitro* transcription/translation system. Especially, the ratio of supercoiled DNA to total plasmid DNA is significantly important. You have to choose more better kits among various commercial products. We guarantee the quality of **DNA-spin™ Plasmid DNA Purification Kit (CAT. 17093)** for *in vitro* transcription/translation kit.
The following tips are technical information for the greatest yields of protein by using **Genelator™** *in vitro* transcription/translation kit.

Choice of Plasmid Kit

There are many commercialized plasmid DNA extraction column kits. However, we only guarantee the quality of plasmid DNA when you use our **iNtRON's DNA-spin™** kit or **Qiagen's (German)** kit.

Do Not Use RNase

Generally, when you use a commercial plasmid DNA extraction kit (column type), you add RNase into lysis solution (DNA-spin™, resuspension buffer) according to the manufacturer's instruction. However, this *in vitro* transcription/translation kit is RNA-based work. **We recommend to not add RNase into lysis solution.** The occurred RNA is not affected in your target protein expression.
If you use RNase-treated plasmid DNA, please add RNase inhibitor during Genelator™ reaction. However, we recommend to prepare again your plasmid DNA.

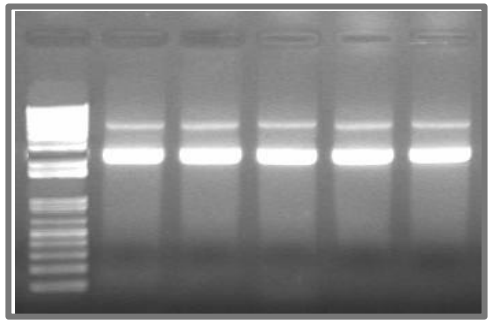
Effect of Chemicals

If you want to extract plasmid DNA manually without using column kit, avoid adding excessive salts or glycerol when extracting DNA. Especially, do not resuspend plasmid DNA with TE (Tris/EDTA) buffer. Instead of TE buffer, you can use distilled H_2O. The EDTA inhibit *in vitro* transcription/translation reaction. The reaction activity may be inhibited by NaCl (<50mM), glycerol (<1%), or by very small amounts of Mg^{2+}, or potassium salts. Our **DNA-spin™ Plasmid DNA Purification Kit** is suitable for *in vitro* transcription/translation system. Any components in DNA-spin™ kit do not inhibit the efficacy of Genelator™ reaction.

Effect of Plasmid DNA Quality

Using various commercialized plasmid DNA extraction kits, we compared the protein synthesis yields. From the results, although the plasmid DNAs show similar yields under UV-transilluminator, the protein yields are very different (under laser-based fluorescence scanning device). So, you should be careful to choose plasmid DNA extraction kit.

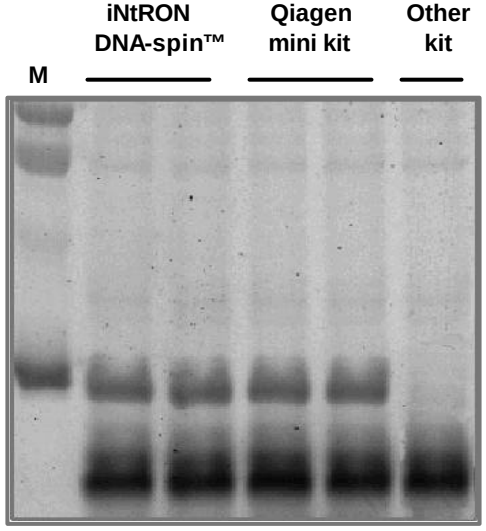
We guarantee only to use our iNtRON's DNA-spin™ kit and Qiagen's spin kit.



- **Plasmid DNA : pD2HM-EGFP**
- **Host *E. coli* : JM110 (*endA*⁺ strain)**

Nicked circles
Supercoiled

- **$OD_{260/280}$: 1.75 ~ 1.85**
- **Adjust to 200ng/μℓ (EGFP)**
- **2μℓ loading at 1.0% Agarose gel**



- **Genelator™ solution : 11.2μℓ (15μℓ rxn.)**
- **EGFP plasmid DNA 2μℓ (200ng/μℓ)**
- **F-Detector™ solution : 1μℓ**
- **1 hr *in vitro* reaction at 37℃**
- **12% SDS-PAGE**
- **Under laser-based fluorescence scanner**

EGFP
29.6 KDa

Important Washing Step in Preparing Plasmid DNA

There are many commercialized plasmid extraction column kits. For an efficient yields of protein *in vitro*, you should be careful to choose those plasmid DNA extraction kit. We recommend again to use our **iNtRON's DNA-spin™ kit**, or **Qiagen's spin kit**.

More important, when you purify a plasmid DNA by using the column kits, you have to process two kinds of Washing Steps. Both iNtRON's DNA-spin™ kit and Qiagen's kit have two kinds of washing solutions including washing buffer A and B. Generally, the washing buffer A step is an optional step useful for removing endonuclease I (*endA*$^+$) enzyme. However, **in this *in vitro* transcription/translation step, you should use those two Washing Buffer A and B although your host *E. coli* is *endA*$^-$ (endonuclease I negative) strain** (refer to right data).

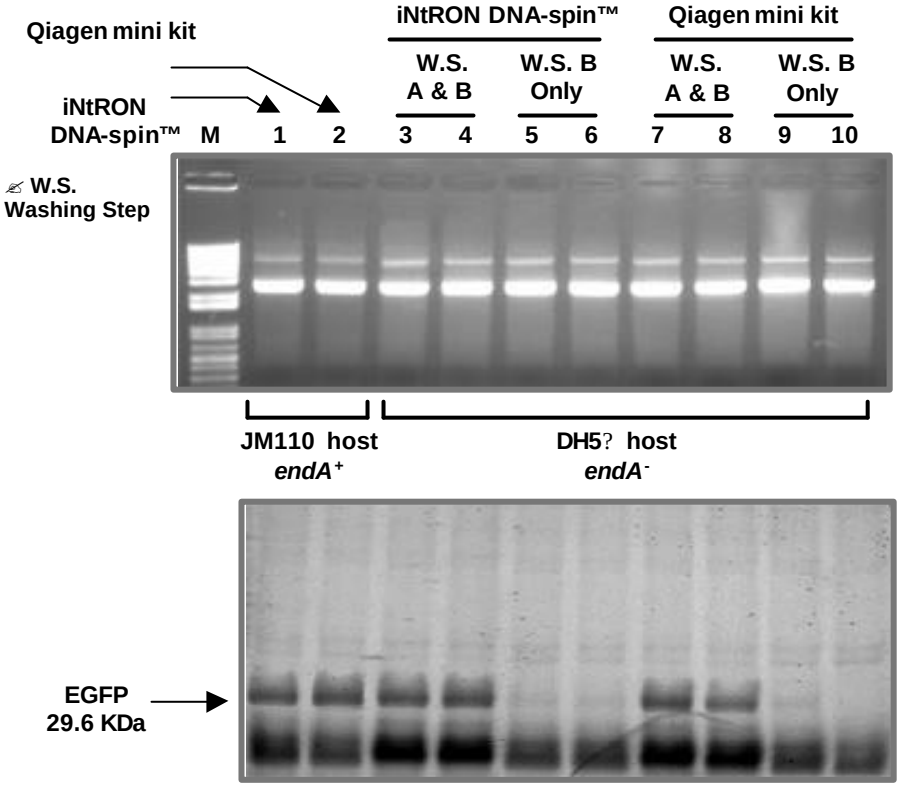
Table. Various *E. coli* Host Strains

endA$^-$	*endA*$^+$
DH1	BL21 (DE3)
DH20	CJ236
DH21	HB101
DH5 α™	JM83
SRB	JM101
XL1-Blue	JM110
XLO	Q358
BJ5183	TB1
JM103	LE392
JM105	MC1061
JM106	NM522 (all NM series strains are *endA*$^+$)
JM107	P2392
JM108	PR700 (all PR series strains are *endA*$^+$)
JM109	RR1
MM294	TG1
Select96™	Y1088 (all Y10 series strains are *endA*$^+$)
SK1590	BMH 71-18
SK1592	ES1301
SK2267	
TOP10	

The *endA*$^-$ strains have no or low endonuclease I activity. The *endA*$^+$ strains have very high endonuclease I activity. Although your strains are *endA*$^-$ strain, please use Washing Buffer A and B solutions.

✍ Washing Step Effects

We have compared the effect of Washing Steps during *in vitro* transcription/translation reaction. We have purified two types of EGFP plasmid DNAs (DH5 α host, *endA*$^-$; JM110 host, *endA*$^+$) with Washing Step A and/or B, or Washing Step A/B, respectively, according to the instruction's manual. From results, you can show that the protein yields are very low when only washing step B was processed during plasmid DNA extraction in DH5 α strain (*endA*$^-$).
The optional washing step (Washing Buffer B) need to get more efficient results in both **iNtRON's DNA-spin™** kit and **Qiagen's** mini kit, although the E. coli strains are endonulcease I negative strain (*endA*$^-$).



- ✍ **Genelator™ solution : 11.2㎕ (15㎕ rxn.)**
- ✍ **EGFP plasmid DNA, each 2㎕ (200ng/㎕)**
- ✍ **F-Detector™ solution : 1㎕**
- ✍ **1 hr *in vitro* reaction at 37℃**
- ✍ **12% SDS-PAGE**
- ✍ **Under laser-based fluorescence scanner**

Genelator™ Protocol for Plasmid DNA

Gene Translator

In Vitro Transcription/Translation Using Plasmid DNA

DESCRIPTION

This protocol is suitable for the *in vitro* production of recombinant proteins from plasmid DNA using the **Genelator™** Mini Kit in a 45 µℓ reaction volume.

The **Genelator™** *in vitro* transcription/translation reaction can be easily scaled–down to as little as 15 µℓ for high-throughput screening in 96-well microplate format. The reagent volumes given in Table 1/2 should be adjusted accordingly. We recommend using polystyrene 96-well plates for high-throughput screening. To avoid evaporation, microplates should be sealed during incubation.

STORAGE RECOMMENDATION

Always avoid multiple freeze-thaw cycles (Do not subject to more than 5 freeze-thaw cycles) or exposure to frequent temperature changes. These fluctuations can greatly alter product stability.

STORAGE & STABILITY

It is recommended the product to be stored at –70 ℃ or –20 ℃ at which it is stable for at least 6 months.

PRECAUTIONS

The **Genelator™** Kit is for laboratory research use only.

This Genelator™ product contains all necessary components for transcription based on T7 or Lac expression system and for translation in a single tube.

MATERIALS

- Genelator™ *in vitro* Transcription/Translation Kit
- F-Detector™ Cell Free Labeling Kit
- Plasmid DNA template (high purity) encoding the protein of interest
- Water-bath, or heating block

IMPORTANT POINTS BEFORE USE

- The *in vitro* transcription/translation system is extremely sensitive to nuclease contamination. Always wear gloves and use RNase- and DNase-free reaction tubes and tips.
- This system is also extremely sensitive to RNase. Please no RNase addition step to general Sol I solution during the purification step of plasmid DNA.
- The plasmid DNA suitable for this system is T7 promoter based or Lac promoter based vector system (we recommend the T7 promoter system for best protein synthesis). If you use other promoter based vector systems such as SP6 promoter, please add an suitable RNA polymerase.
- You can easily check the expression of protein by using conventional SDS-PAGE analysis using **F-Detector™** Cell Free Labeling Kit (CAT. 12021/12022). You can easily determine your protein expression on UV transilluminator.
- Except for the actual transcription-translation incubation (37 ℃), all handling steps should be carried out on ice (important).
- The Master Mix in the **Genelator™** Kit is sensitive to multiple freeze–thaw cycles. The Master Mix are provided as individual aliquots in single tubes. Once thawed, use the Master Mix within 4 hours. Do not refreeze and thaw more than five times.
- To determine the background level of protein synthesis, always include a no-template control reaction (negative control) in your experiment. Also, to determine the success of reaction, you can perform same reaction with EGFP positive control DNA (freely supplied EGFP vector DNA). The qualitative EGFP estimates could be made using a UV-lamp (360 nm) in a dark room.
- The functionality of the kit can be checked by performing a positive control reaction using Genelator™ Positive-Control DNA that encodes the 27 KDa EGFP protein with a C-terminal 6xHis tag.
- The recommended incubation temperature for protein synthesis is 37 ℃, but lower incubation temperatures may improve protein solubility in some cases.

iNtRON Related Spin Series Products

DNA-spin™	MEGA-spin™	PCRquick-spin™
Plasmid DNA from *E. coli*	Elute DNA from Agarose	Clean-up Kit after PCR
CAT. 17093	CAT. 17183	CAT. 17202

Cat. 12011 >> 25 ea. X 45μℓ Rxn. (Mini Kit)

Protocol : Cell Free Protein Synthesis Using Plasmid DNA

PROCEDURE (*in vitro* Transcription/Translation Reaction)

1. Remove the tube containing Genelator™ Master Mix from the freezer and allow it to thaw briefly Genelator™ Master Mix at 37℃ water bath (less than 60 sec.), or by warming by hand. It is best to thaw briefly by hand-warming.

2. After thawing, briefly spin down the tube for 1~5 sec.; immediately upon thawing, place the tube on ice. If required, allow the tube on ice bath to cool to 2~4℃.

The following 3 and 4 steps should be carried out on ice bath.

[NOTE] The Genelator™ Kit is adjusted into total 45μℓ reaction volume. However, you can easily scaled–down to 15μℓ reaction volume. In this case, aliquot upper thawing Genelator™ Master Mix into new tube by 11.2μℓ each. If so, you can get 3 tubes suitable for 15μℓ reaction with one Genelator™ tube. In a reference, One Genelator™ tube contains 33.6μℓ of Master Mix. Please refer to Table 1.

3. Add 0.5~2.0μg plasmid DNA to each reaction tube. Add 2.5μℓ Genelator™ positive control DNA(200ng/μℓ EGFP plasmid DNA, 5.0μℓ x 200ng = 1.0μg) to the positive-control reaction tube. Do not add any DNA to the no-template control reaction tube (negative control).

[NOTE] The amount of plasmid DNA added to each 45μℓ *in vitro* transcription and translation reaction should be 0.5~1.0μg for plasmids up to 5 kb in size, or 1.0~2.0μg for plasmids >5 kb.

4. Bring the final reaction volume to 45μℓ with RNase-free water (supplied). Table 1 provides a pipetting scheme for Genelator™ *in vitro* Transcription and Translation Mini Kit.

[NOTE] If you use **F-Detector™ Cell Free Labeling Kit** or Radioisotope-labeled amino acid, you must firstly add 3μℓ of F-Detector™ Solution (1μℓ addition per 15μℓ reaction; refer to F-Detector™ Kit), or approximate Labeled amino acid, before filling up with dH_2O by 45μℓ volume, respectively.
And more, avoid using low purity DNA. Assure that the $A_{260/280}$ ratio is at least >1.7. This system is also extremely sensitive to RNase. Please no RNase addition step to general Sol I solution during the purification step of plasmid DNA.

5. Mix and centrifuge briefly to collect the reactions in the bottom of the tubes.

6. Incubate the reconstituted reaction mixture for an appropriate time (usually 60 min.) at 37℃ (lower temperatures may be used for proteins that tend to aggregate).
(optional) To achieve optimal distribution of reaction components, samples should be shaken in a thermomixer or water-bath shaker.

7. Stop the reaction by placing on ice, and centrifuge briefly to collect the reactions in the bottom of the tubes.

8. Proceed with sample analysis such as SDS-PAGE or Functional Analysis.

Table 1. Pipetting Scheme for Setup of Genelator™ *in vitro* Transcription & Translation Mini Kit Reaction Using Plasmid DNA as a Template

Components	15μℓ Reaction Volume			45μℓ Reaction Volume		
	User Sample	Positive control	Negative control	User Sample	Positive control	Negative control
Genelator™ Master Mix	11.2μℓ	11.2μℓ	11.2μℓ	33.6μℓ	33.6μℓ	33.6μℓ
DNA Template	0.1μg ~ 0.5μg	<2.0μℓ(2)	X	0.5μg ~ 2.0μg	<5.0μℓ(2)	X
F-Detector™ Kit (1)	(1.0μℓ)	(1.0μℓ)	(1.0μℓ)	(3.0μℓ)	(3.0μℓ)	(3.0μℓ)
RNase-Free Water	Add to 15μℓ volume	1.8μℓ (0.8μℓ)	3.8μℓ (2.8μℓ)	Add to 45μℓ volume	6.4μℓ (3.4μℓ)	11.4μℓ (8.4μℓ)

(1) You can easily check the expression of protein on UV transilluminator, or Laser-based fluorescence scanning device by using F-Detector™ Kit (Cat. 12021/12022).
(2) Genelator™ Positive-Control DNA (EGFP, 200ng/μℓ) freely supplied with the kit

Protocol : Cell Free Protein Synthesis Using Plasmid DNA

PROCEDURE (*in vitro* Transcription/Translation Reaction)

1. Remove the tube containing Genelator™ Master Mix from the freezer and allow it to thaw briefly Genelator™ Master Mix at 37℃ water bath (less than 2~3 min).

2. After thawing, briefly spin down the tube for 1~5 sec.; immediately upon thawing, place the tube on ice. If required, allow the tube on ice bath to cool to 2~4℃.

The following 3 and 4 steps should be carried out on ice bath.

[NOTE] The Genelator™ kit (Cat. No. 12012) is adjusted into total 900㎕ reaction volume. One tube contains 672㎕ of Master Mix .

3. Add 10㎍~40㎍ plasmid DNA to each reaction tube. Add 40㎍ Genelator™ positive control DNA (200ng/㎍ EGFP plasmid DNA, 40㎕ x 200ng = 8㎍) to the positive-control reaction tube. Do not add any DNA to the no-template control reaction tube (negative control).

[NOTE] The amount of plasmid DNA added to each 900㎕ *in vitro* transcription and translation reaction should be 5~20㎍ for plasmids up to 5 kb in size, or 20~40㎍ for plasmids >5 kb.

4. Bring the final reaction volume to 900㎕ with RNase-free water (supplied). Table 2 provides a pipetting scheme for Genelator™ *in vitro* Transcription and Translation Midi Kit.

[NOTE] If you use **F-Detector™ Cell Free Labeling Kit** or Radioisotope-labeled amino acid, you must firstly add 60㎕ of F-Detector™ Solution (refer to **F-Detector™ Kit**), or approximate Labeled amino acid, before filling up with dH_2O by 900㎕ volume, respectively.
And more, avoid using low purity DNA. Assure that the $A_{260/280}$ ratio is at least >1.7. This system is also extremely sensitive to RNase. Please no RNase addition step to general Sol I solution during the purification step of plasmid DNA.

5. Mix and centrifuge briefly to collect the reactions in the bottom of the tubes.

6. Incubate the reconstituted reaction mixture for an appropriate time (usually 60 min.) at 37℃ (lower temperatures may be used for proteins that tend to aggregate).
(optional) To achieve optimal distribution of reaction components, samples should be shaken in a thermomixer or water-bath shaker.

7. Stop the reaction by placing on ice, and centrifuge briefly to collect the reactions in the bottom of the tubes.

8. Proceed with sample analysis such as SDS-PAGE or Functional Analysis.

Table 2. Pipetting Scheme for Setup of Genelator™ *in vitro* Transcription & Translation Midi Kit Reaction Using Plasmid DNA as a Template

Components	900㎕ Reaction Volume		
	User sample	Positive control	Negative control
Genelator™ Master Mix	672㎕	672㎕	672㎕
DNA Template	5㎍ ~ 40㎍	<40㎕[2]	X
F-Detector™ Kit [1]	(60㎕)	(60㎕)	(60㎕)
RNase-Free Water	Add to 900㎕ volume	188㎕ (128㎕)	228㎕ (168㎕)

[1] You can easily check the expression of protein on UV transilluminator, or Laser-based fluorescence scanning device by using F-Detector™ Kit (Cat. 12021/12022).
[2] Genelator™ Positive-Control DNA (EGFP, 200ng/㎕) freely supplied with the kit

Protocol : Analysis of *in vitro* Synthesized Protein

After *in vitro* transcription/translation reaction, you can analyze the reaction mixture in gel electrophoresis or functional analysis directly.

PROCEDURE (For SDS-PAGE Analysis)

This Gel Analysis Protocol is general iNtRON SDS-PAGE analysis method. If you have another protocol for SDS-PAGE analysis, there is no problem for analyzing protein.

1. When *in vitro* protein synthesis reaction is complete (or at any desired time point), remove 3~5㎕ aliquot. The remainder of the reaction may be stored at -20℃ or –70℃ .

[NOTE] If you want to analyze proteins more than 10㎕ among above samples, we suggest precipitating the samples with acetone prior to the addition of SDS gel sample buffer (conventional acetone precipitation) .

2. Add 20㎕ of 1? SDS gel sample buffer to sample tube.

3. Thoroughly vortex for mixing and briefly centrifuge.

4. Heat for 2~5 min at 95℃ using heat block to denature the proteins.

[NOTE] Occasionally, denaturation at 100℃ results in the formation of large molecular weight species that do not migrate well on gels. Denaturing at 80-90℃ avoids formation of these large molecules. (**optional** : centrifuge at 13,000 rpm for 2~15 min without cooling to pellet the insoluble material.)

5. Carefully apply the supernatant of the denatured sample onto the gel (usually 12% SDS-PAGE or SDS-urea-PAGE gel).

6. Typically, electrophoresis is carried out at constant voltage of 50V in the stacking gel, and 100~150V in the separating gel. Electrophoresis is usually performed until Bromophenol Blue dye has run off the bottom of the gel.

7. Detect the synthesized protein with Coomassie Brilliant Blue (CBB) staining, fluorescence detection method using UV transilluminator or fluorescent scanner, or autoradiography.

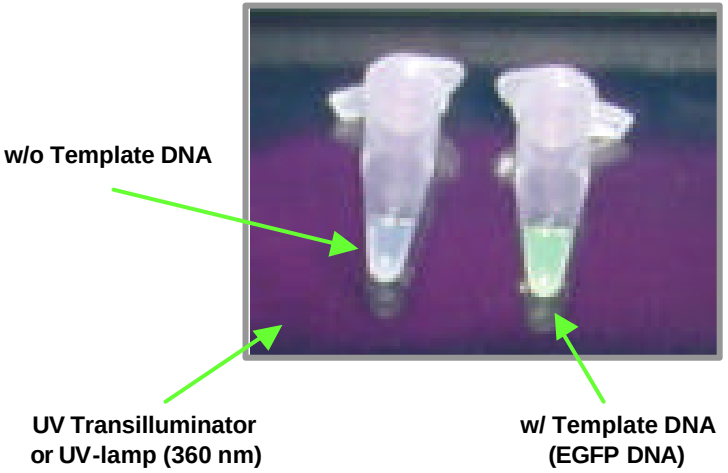
PROCEDURE (For Functional Analysis of *in vitro* Synthesized Protein)

You can perform directly functional analysis of *in vitro* synthesized protein according to the characteristics of your protein.

1. Centrifuge at 11,000 rpm for 10 min without cooling to obtain a soluble protein fraction (**optional**).

2. Use this supernatant directly in functional analysis such as *in vitro* bioassay or enzymatic activity assay.

Fig 3. Fluorescence Detection of EGFP protein.
UV visualization of enhanced green fluorescent protein (EGFP) produced using Genelator™ *in vitro* Transcription/Translation Mini Kit. In vitro protein synthesis without DNA template (***left tube***), and with EGFP positive-control DNA (***right tube***).



[cf] The GFP needs molecular oxygen to form the fluorophore post-translationally. The yield of properly-folded fluorescent GFP is further increased by storing the reaction solution after the expression of 24 hours at 4℃. After 24 hours of storing at 4℃, the maturation is almost completed.

SDS-PAGE & Fluorescence Scanning

Genelator™ Mini/Midi DESCRIPTION

Using **Genelator™** ***in vitro*** **Transcription/Translation Mini/Midi Kit**, target protein could be produced as a soluble form in large-scale quantity compared to conventional commercial cell-free translation kit. The **Genelator™ Kit** is a simple one-pot type system including T7 RNA polymerase and is suitable for the production of aggregation-prone protein originating from eukaryote as a soluble form. And **Genelator™ Kit** serves as an alternative method for *in vivo* expression, especially for toxic proteins and/or protease sensitive proteins.

Genelator™ DATASHEET

✍ **EPO (Erythropoietin) Production by Genelator™ Kit**

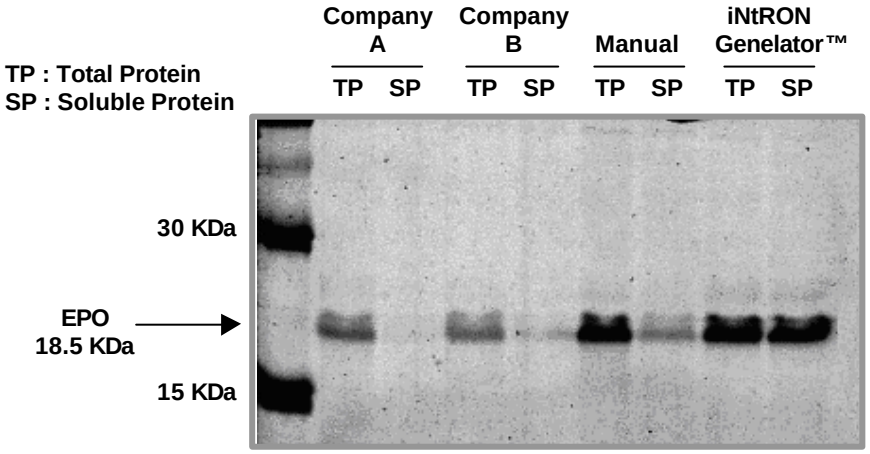


Fig 4. SDS-PAGE and Fluorescent Scanning of Erythropoietin (EPO)
The EPO protein was produced in several cell-free protein synthesis systems and labeled using F-Detector™ Kit. EPO is a eukaryotic protein which is produced in biologically inactive and inclusion body when it is expressed in bacteria.
TP : Total protein produced via various Cell-Free System
SP : Soluble protein (in the supernatant after centrifugation ; 10,000xg, 10min.) produced in various Cell-Free System
Company A : commercial cell-free protein synthesis kit
Company B : commercial cell-free protein synthesis kit
Manual : conventional cell-free protein synthesis method prepared by us
Genelator™ : Genelator™ *in vitro* Transcription/Translation Mini Kit

✍ **CAT and GFP Production by Genelator™ Kit**
(CAT, Chloramphenicol Acetyl Transferase; GFP, Green Fluorescent Protein)

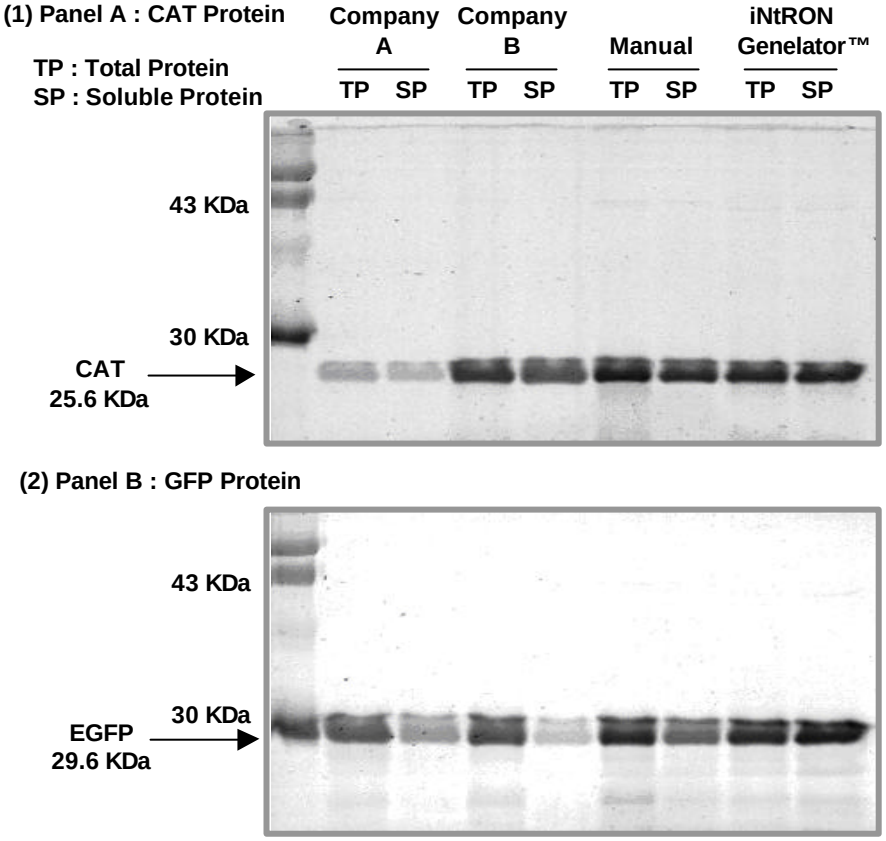


Fig 5. SDS-PAGE and Fluorescent Scanning of Chloramphenicol Acetyl Transferase (CAT, panel A) and Green Fluorescent Protein (GFP, panel B)
The CAT is a cytoplasmic soluble protein, and GFP protein is a natural fluorescence detectable protein. The translated proteins were labeled using F-Detector™ Kit.
TP : Total protein produced via various Cell-Free System
SP : Soluble protein (in the supernatant after centrifugation ; 10,000xg, 10min.) produced in various Cell-Free System
Company A : commercial cell-free protein synthesis kit
Company B : commercial cell-free protein synthesis kit
Manual : conventional cell-free protein synthesis method prepared by us
Genelator™ : Genelator™ *in vitro* Transcription/Translation Mini Kit

SDS-PAGE & Coomassie Brilliant Blue Staining

✍ SDS-PAGE and Coomassie Brilliant Blue Staining (DHFR, CAT, and EGFP)

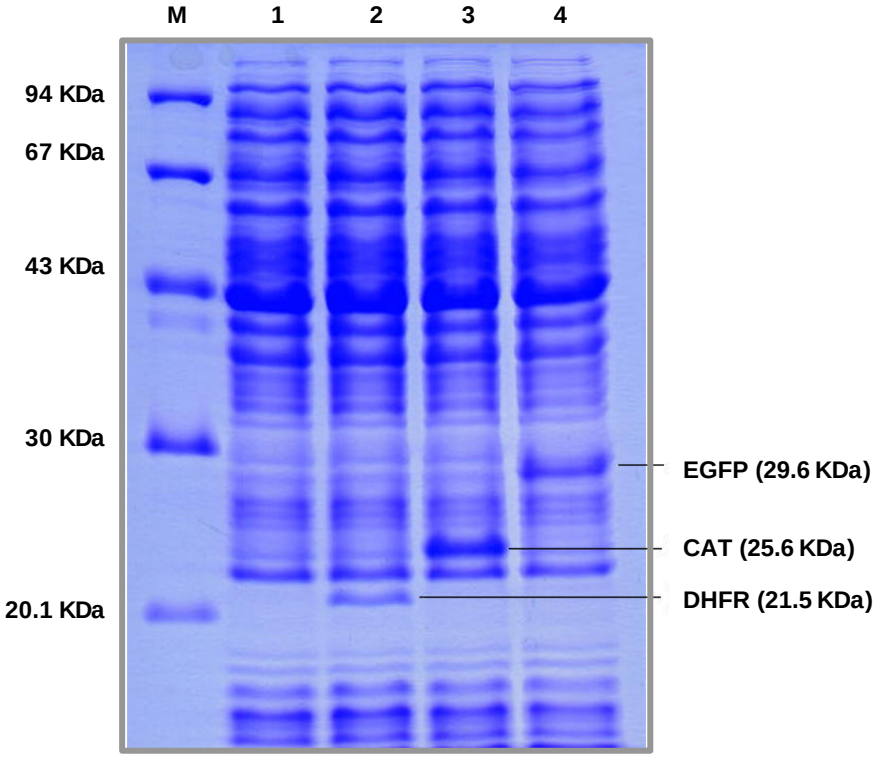


Fig 6. SDS-PAGE and Coomassie Brilliant Blue (CBB) Staining of Three Kinds of Proteins Synthesized by Genelator™ Kit

After synthesizing proteins with Genelator™ kit, the SDS-PAGE (12%) was performed, and the gel was stained with CBB according to standard protocol.

Lane 1 : Negative control (No DNA template)
Lane 2 : DHFR (21.5 KDa, Dihydrofolate reductase) plasmid DNA addition
Lane 3 : CAT (25.6 KDa) plasmid DNA addition
Lane 4 : EGFP (29.6 KDa) plasmid DNA addition

✍ SDS-PAGE and Coomassie Brilliant Blue Staining (β-gal and GroEL)

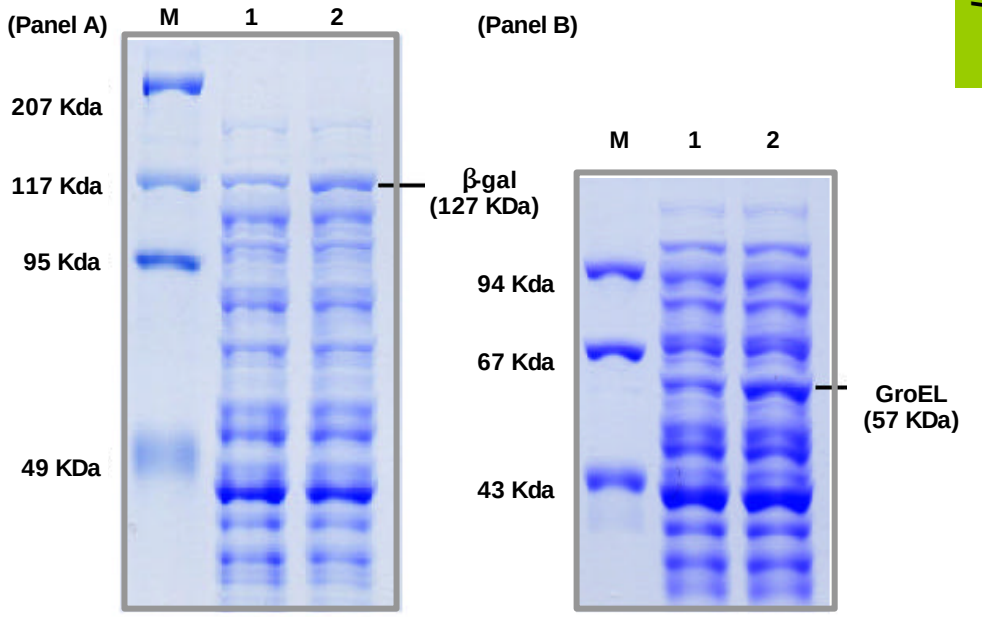


Fig 7. SDS-PAGE and Coomassie Brilliant Blue (CBB) Staining of Two Kinds of Proteins Synthesized by Genelator™ Kit

After synthesizing proteins with Genelator™ kit, the SDS-PAGE was performed, and the gel was stained with CBB according to standard protocol.

[PANEL A]
Lane 1 : Negative control (No DNA template)
Lane 2 : β-gal (127 KDa, β-galactosidase) plasmid DNA addition

[PANEL B]
Lane 1 : Negative control (No DNA template)
Lane 2 : GroEL (57 KDa, Chaperonin) plasmid DNA addition

Co-Expression of Multiple Proteins in a Single Reaction

✍ SDS-PAGE and Coomassie Brilliant Blue Staining

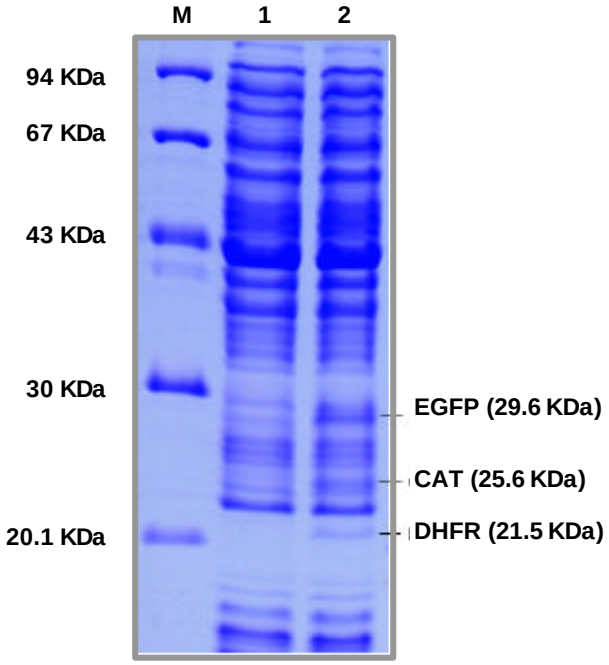


Fig 8. Co-expression of DHFR, CAT and EGFP in a Single Reaction Tube
For co-expression of multiple proteins, the cell free protein synthesis reaction was performed by using Genelator™ kit in a single reaction tube with DHFR, CAT, and EGFP plasmids. After *in vitro* synthesizing proteins, SDS-PAGE (12%) and CBB staining were performed.
Lane 1 : Negative control (No DNA template)
Lane 2 : EGFP, CAT, and DHFR DNAs addition in a single reaction tube

Other Products Information

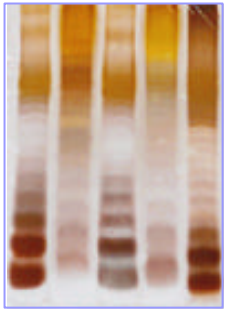
::: LPS (Lipopolysaccharide) Extraction Kit — CAT. 17141

✍ **LPS Extraction from Bacteria**
✍ **Distribution of Bacteria by Patterns of the Carbohydrate Chain Length**

LPS (lipopolysaccharide) triggers a cascade of immunostimulatory and toxic pathophysiological events, including peripheral vascular collapse by releasing endotoxin initiate septic shock. The iNtRON LPS Extraction Kit is the first commercially product for extracting LPS from bacteria. LPS Extraction Kit is designed for rapid and convenient extraction of LPS from bacterial cells.

Bacteria Harvest
↓
Add Lysis Buffer
↓
Add Chloroform (not supplied)
↓
Centrifuge
↓
Add Purification Buffer
↓
Centrifuge
↓
Wash Pellet / Dissolve

LPS Pattern Analysis
PAGE & Sillver Staining



Just < 60 min
Very Convenient

::: i-StarMaster™ PCR Master Mix Kit (96 tubes : 8? 12 strips, 20? l rxn.)

✍ **High Amplification & Sensitivity** — **CAT. 25241**
✍ **Multiplex PCR / Hot Start PCR / PCR Diagnosis**

Fig 1. Sensitivity Assay
Panel A : company A
Panel B : iNtRON
M : DNA marker,
Lane 1 : 50ng template DNA
Lane 2 : diluted DNA

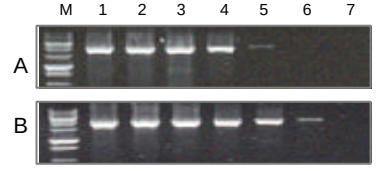
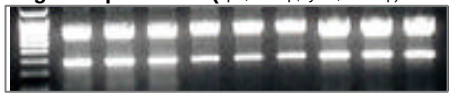


Fig 2. Duplex PCR (irp2, 800bp; fyuA, 300 bp)



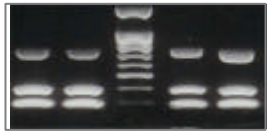
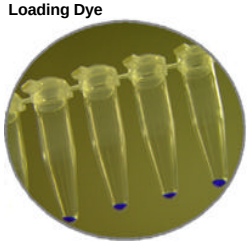


Fig 3. Triplex PCR
(fyuA, 780bp; tsh, 420bp; Irp2, 280bp)

CONTENTS
✍ **Master Mix Solution**
i-StarTaq™ 10x PCR Buffer
dNTP (10mM), $MgCl_2$
Chemical Stabilizer

✍ **Master Tube (lyophilized)**
i-StarTaq™ DNA Polymerase
Enzyme Stabilizer
Loading Dye



Very Convenient !
Just Add Template DNA & Primers

Genelator™ Protocol for PCR Product

Gene Translator

In Vitro Transcription/Translation Using PCR Product

DESCRIPTION

This protocol is suitable for the *in vitro* production of recombinant proteins from PCR products using the Genelator™ Mini Kit in a 45 ㎕ reaction volume. The PCR reaction must be performed using primers specific for the T7 promoter and terminator regions. The Genelator™ *in vitro* transcription/translation reaction can be easily scaled–down to as little as 15 ㎕ for high-throughput screening in 96-well microplate format. The reagent volumes given in Table 3 should be adjusted accordingly. We recommend using polystyrene 96-well plates for high-throughput screening. To avoid evaporation, microplates should be sealed during incubation.

A major advantage of this method is that it does not require sub-cloning, bacterial transfection, or fermentation, and is consequently very rapid. High-throughput protein expression screening based on cell-free protein synthesis is useful for large-scale proteomic efforts aimed at identifying and characterizing expressed proteins.

This Genelator™ product contains all necessary components for transcription based on T7 or Lac expression system and for translation in a single tube.

MATERIALS

■ Genelator™ *in vitro* Transcription/Translation & F-Detector™ Cell Free Labeling Kits
■ PCR template encoding the protein, which must contain T7 promoter and terminator regions.
■ Water-bath, or heating block

IMPORTANT POINTS BEFORE USE

■ The *in vitro* transcription/translation system is extremely sensitive to nuclease contamination. Always use RNase- and DNase-free reaction tubes and tips.
■ PCR reaction must be performed using primers specific for the T7 promoter and terminator region. Please contact us if you have any questions.
■ Use the PCR product directly for *in vitro* protein synthesis, or after purification with **PCRquick-spin™** (Cat. 17202), which can be used to remove potentially inhibitory primer dimers. Do not purify DNA-fragments over agarose gel, because this treatment inhibits *in vitro* protein synthesis.
■ You can easily check the expression of protein by using conventional SDS-PAGE analysis via **F-Detector™ Cell Free Labeling Kit**. You can determine your protein expression on UV transilluminator.
■ Except for the actual transcription-translation incubation (37℃), all handling steps should be carried out on ice (important).
■ The Master Mix in the Genelator™ Kit is sensitive to multiple freeze–thaw cycles. Master Mix are provided as individual aliquots in single tubes. Once thawed, use Master Mix within 4 hours. Do not refreeze and thaw more than five times.
■ To determine the background level of protein synthesis, always include a no-template control reaction (negative control) in your experiment. Also, to determine the success of reaction, you can perform same reaction with EGFP positive control DNA (supplied EGFP vector DNA). The qualitative EGFP estimation could be made using a UV-lamp (360 nm) in a dark room.
■ The functionality of the kit can be checked by performing a positive control reaction containing a control using Genelator™ Positive-Control DNA that encodes the 27 kDa EGFP protein with a C-terminal 6xHis tag.
■ The recommended incubation temperature for protein synthesis is 37 ℃, but lower incubation temperatures may improve protein solubility in some cases.

PROCEDURE (*IN VITRO* TRANSCRIPTION/TRANSLATION REACTION)

1. Remove the tube containing Genelator™ Master Mix from the freezer and allow it to thaw briefly Genelator™ Master Mix at 37℃ water bath (less than 60 sec.), or by warming by hand. It is best to thaw briefly by hand-warming.

2. After thawing, briefly spin down the tube for 1~5 sec.; immediately upon thawing, place the tube on ice. If required, allow the tube on ice bath to cool to 2~4℃.

The following 3 and 4 steps should be carried out on ice bath.

[NOTE] The Genelator™ kit is adjusted into total 45㎕ reaction volume. However, you can easily scaled–down to 15㎕ reaction volume. In this case, aliquot upper thawing Genelator™ Master Mix into new tube by 11.2㎕ each. If so, you can get 3 tubes suitable for 15㎕ reaction with one Genelator™ tube. In a reference, One Genelator™ tube contains 33.6㎕ of Master Mix. Please refer to Table 3.

3. Add 0.5~2.0㎍ PCR product (5~10㎕)each reaction tube. Do not add any DNA to the no-template control reaction tube (negative control).

4. Bring the final reaction volume to 45㎕ with RNase-free water (supplied).

5. Mix and centrifuge briefly to collect the reactions in the bottom of the tubes.

6. Incubate the reconstituted reaction mixture for an appropriate time (usually 60 min.) at 37℃ (Lower temperatures may be used for proteins that tend to aggregate).
(optional) To achieve optimal distribution of reaction components, samples should be shaken in a thermomixer or water-bath shaker.

7. Stop the reaction by placing on ice, and centrifuge briefly to collect the reactions in the bottom of the tubes.

8. Proceed with sample analysis such as SDS-PAGE or Functional Analysis.

Table 3. Pipetting Scheme for Setup of Genelator™ *in vitro* Transcription & Translation Mini Kit Reaction Using PCR Product as a Template

Components	15㎕ Reaction Volume			45㎕ Reaction Volume		
	User Sample	Positive control	Negative control	User Sample	Positive control	Negative control
Genelator™ Master Mix	11.2㎕	11.2㎕	11.2㎕	33.6㎕	33.6㎕	33.6㎕
DNA Template	0.1㎍ ~ 0.5㎍	<2.0㎕[2]	X	0.5㎍ ~ 2.0㎍	<5.0㎕[2]	X
F-Detector™ Kit [1]	(1.0㎕)	(1.0㎕)	(1.0㎕)	(3.0㎕)	(3.0㎕)	(3.0㎕)
RNase-Free Water	Add to 15㎕ volume	1.8㎕ (0.8㎕)	3.8㎕ (2.8㎕)	Add to 45㎕ volume	6.4㎕ (3.4㎕)	11.4㎕ (8.4㎕)

[1] You can easily check the expression of protein on UV transilluminator, or Laser-based fluorescence scanning device by using F-Detector™ Kit (Cat. 12021/12022).
[2] Genelator™ Positive-Control DNA (EGFP, 200ng/㎕) freely supplied with the kit

SDS-PAGE & Fluorescence Scanning

✍ SDS-PAGE and Fluorescent Scanning of Proteins Using PCR Product

To implement the expression-screening procedure in a high-throughput format, polymerase chain reactions (PCRs) were performed using primers specific for the T7 promoter and terminator regions. A major advantage of this method is that it does not require sub-cloning, bacterial transfection, or fermentation, and is consequently very rapid. High-throughput protein-expression screening based on cell-free protein synthesis is useful for large-scale proteomic efforts aimed at identifying and characterizing expressed proteins.
The translated proteins were labeled using F-Detector™ Kit. The presence of an 18 KDa endogenous fluorescent band can be removed by treatment with RNase A (refer to F-Detector™ Kit protocol).

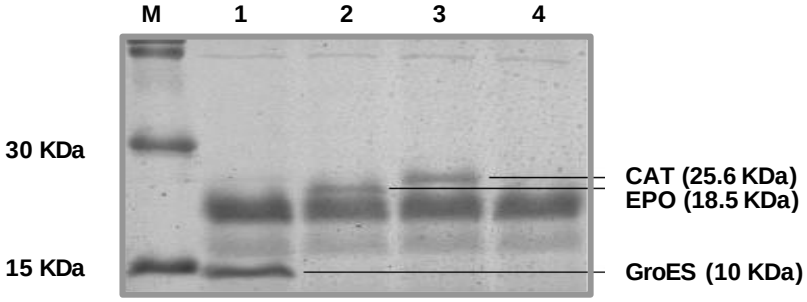


Fig 9. SDS-PAGE and Fluorescence Scanning of Three Kinds of Proteins
The cell free protein synthesis reaction was performed using PCR products including CAT, EPO, or GroES genes. After synthesizing proteins, SDS-PAGE (12%) and laser-based fluorescence scanning were performed.
Lane 1 : GroES (10 KDa, Chaperonin)
Lane 2 : EPO (18.5 KDa, Erythropoietin)
Lane 3 : CAT (25.6 KDa, Chloramphenicol acetyl trasnferase)
Lane 4 : Negative control (No PCR product)

Product Background

F-Detector™ Cell Free Labeling Kit

Many protein modification systems either post-translational or post-synthetic modification have been developed. But, these methods have not been able to label the growing nascent proteins selectively during their synthesis for detection and are unsuitable for selective labeling of the produced protein in a pre-existing protein mixture. A variety of techniques have been investigated for labeling the nascent protein during their synthesis. There are two major classes of commonly used detection methods. The translated proteins are detected based on radioactivity or biotin moiety. The two complementary detection systems are useful for screening of expressed proteins. One of the most common methods used in the detection of proteins synthesized or characterized using *in vitro* translation systems is the incorporation of radioactively labeled amino acids such as [^{35}S]Methionine or [^{14}C]Leucine. Safety, regulatory issues, waste disposal, and lengthy exposure times are the primary drawbacks associated with the use of radioactivity. The use of radioactivity is undesirable for routine laboratory applications as well as for the automated analysis of samples. As with radioactive incorporation, in the method based on biotin moiety, reactions containing proteins are resolved on SDS-PAGE gels and the proteins must be electroblotted to a solid matrix such as nitrocellulose or PVDF membranes. And electroblotted proteins can be detected non-isotopically using either colorimetric or chemiluminescent detection reagents.

F-Detector™ Cell Free Labeling Kit serves a sensitive, non-isotopic, fluorescence-based method for the detection of nascent proteins directly in gels with high signal-to noise ratios after *in vitro* synthesis. A fluorescent technique using **F-Detector™ Cell Free Labeling Kit** offers a simplified alternative method that dramatically reduces the time required to obtain data due to the rapid detection of the protein bands immediately after electrophoresis without any downstream processing and eliminates manipulations associated with radioactivity (*i.e.*, gel fixing and drying or transferring to a membrane). It also eliminates the requirement for electroblotting associated with other non-isotopic technologies based on the biotin moiety.

Under the optimal conditions, fluorescent bands from nanogram levels of *in vitro*-produced proteins could be detected directly from gels using a conventional UV-transilluminator. Higher sensitivity (~100-fold) can be obtained using a laser-based fluorescent gel scanner. Fluorescent marker in **F-Detector™ Cell Free Labeling Kit** can be excitable over a wide range of wavelengths for detection with both an UV-transilluminator (gel documentation system) and a laser-based fluorescent gel scanner.

The ability to rapidly synthesize nascent proteins containing a fluorescent reporter group facilitates many biotechnological applications including functional analysis of gene products, drug discovery, and mutation screening. For example, nascent proteins labeled with fluorophore should be suitable for detection by laser-induced fluorescence (LIF) in conjunction with capillary electrophoresis (CE). This technology can be also be used to rapidly screen for protein truncation products which are produced from defective mutant genes or for the interaction between a target *in vitro* produced protein and other molecules such as in the screening for drug candidates. Other possible application of **F-Detector™ Cell Free Labeling Kit** include *in vitro* expression cloning (IVEC), *in vitro* selection of proteins, and functional gene screening based on an antisense display. **F-Detector™ Cell Free Labeling Kit** could be readily used for high-throughput screening to identify protein expression and examine its function. iNtRON

F-Detector™ Cell Free Labeling Kit

CAT. NO. 12021	CAT. NO. 12022
Total 60μℓ (<3μℓ per Genelator™ 45μℓ rxn.) (1) F-Detector™ Fluorescent tRNA one tube containing 60μℓ mixture (2) Handbook Guide	Total 300μℓ (<3μℓ per Genelator™ 45μℓ rxn.) (1) F-Detector™ Fluorescent tRNA 5 tubes containing 60μℓ mixture (2) Handbook Guide

F-Detector™ Protocol

Fluorescence Detector

Non-Radioisotope Labeling : F-Detector™

DESCRIPTION

F-Detector™ Cell Free Labeling Kit serves a sensitive, non-isotopic, fluorescence-based method for the detection of nascent proteins directly in gels with high signal-to noise ratios after *in vitro* synthesis. A fluorescent technique using **F-Detector™ Kit** offers a simplified alternative method that dramatically reduces the time required to obtain data due to the rapid detection of the protein bands immediately after electrophoresis without any downstream processing and eliminates manipulations associated with radioactivity (*i.e*., gel fixing and drying or transferring to a membrane). It also eliminates the requirement for electroblotting associated with other non-isotopic technologies based on the biotin moiety.

STORAGE RECOMMENDATION

Always avoid multiple freeze-thaw cycles (do not subject to more than 5 freeze-thaw cycles) or exposure to frequent temperature changes. These fluctuations can greatly alter product stability. Dispense the product into smaller aliquots as needed.

STORAGE & STABILITY

It is recommended the product to be stored at –70℃ or –20℃ at which it is stable for at least 6 months.

PRECAUTIONS

The F-Detector™ and Genelator™ Kit is for laboratory research use only.

KIT CONTENTS

This product contains a fluorescent modified tRNA. This fluorescent modified tRNA allows the fluorescent detection of proteins synthesized in cell-free protein synthesis systems. Fluorescent dye used for this labeling is 5(6)-carboxyfluorescein [5(6)-FAM; Excitation maximum: 493nm; Emission maximum: 520nm; Molar extinction coefficient: 78,600 $cm^{-1}M^{-1}$] (Figure 11).

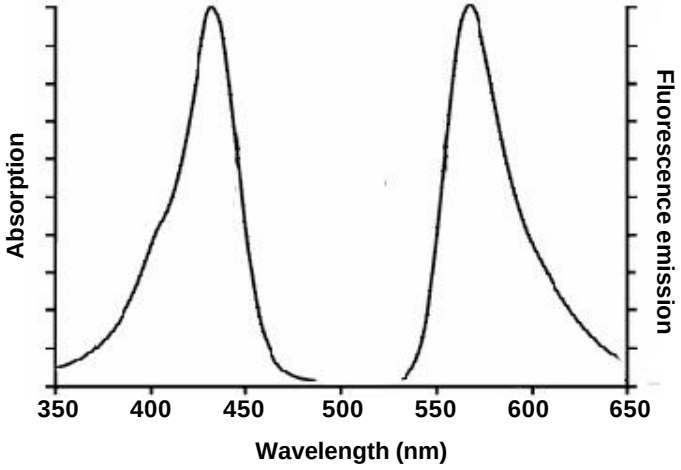
MATERIALS

- Genelator™ *in vitro* Transcription/Translation Kit
- F-Detector™ Cell Free Labeling Kit
- Plasmid DNA template (high purity) encoding the protein of interest
- Water-bath, or heating block

IMPORTANT POINTS BEFORE USE

- The F-Detector™ Kit is extremely sensitive to nuclease contamination. Always use RNase- and DNase-free reaction tubes and pipet tips.
- The fluorescent modified tRNA in F-Detector™ Kit is sensitive to multiple freeze-thaw cycles. The kit is provided as 60μℓ individual aliquots in a single tube. Once thawed, use F-Detector™ Solution within 2 hours. Do not refreeze and thaw more than five times. Dispense the product into smaller aliquots as needed.
- This system is also extremely sensitive to RNase. Please no RNase addition step to general Sol I solution during the purification step of plasmid DNA.
- The plasmid DNA suitable for this system is T7 promoter based or Lac promoter based vector system. If you use other promoter based vector systems such as SP6 promoter, please add an suitable RNA polymerase.
- Except for the actual transcription-translation incubation (37℃), all handling steps should be carried out on ice (important).
- The recommended incubation temperature for protein synthesis is 37℃, but lower incubation temperatures may improve protein solubility in some cases.

Fig 10. Absorption & Fluorescence Emission Spectra of 5(6)-Carboxyfluorescein



Notes For F-Detector™ Kit

As described, **F-Detector™ Cell Free Labeling Kit** serves a sensitive, non-isotopic, fluorescence-based method for the detection of nascent proteins directly in gels *in vitro* protein synthesis.
You can use this **F-Detector™ Kit** in various *in vitro* transcription/translation systems from other companies. This labeling kit enables to easily detect your targeted protein without using an radioisotope. During *in vitro* transcription/translation reaction, you may co-incubate after adding an appropriate amount of **F-Detector™** solution. After finishing SDS-PAGE, you can easily detect your target protein synthesis on UV-transilluminator, gel documentation system, or laser-base fluorescence scanning device.
Especially, when you use an fluorescent gene such as EGFP gene, you can also check the fluorescence expression in a reaction tube without SDS-PAGE.

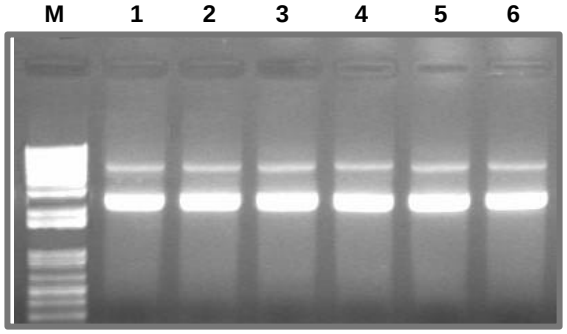
Adding Amounts of F-Detector™

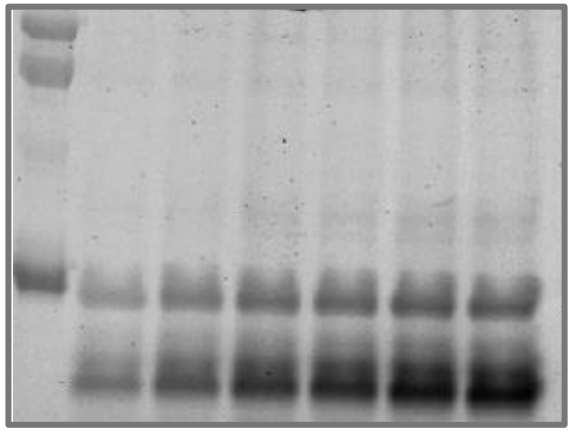
We recommend to add only 1㎕ of F-Detector™ solution per 15㎕ *in vitro* transcription/translation reaction. You may add proportionally more F-Detector™ solution according to your reaction volume. The F-Detector™ solution does not affect to protein synthesis yield itself during *in vitro* transcription/translation reaction.
More and more adding F-Detector™ solution to your reaction, the signal of expression may be only strong.

The following data shows the signal density according to the amounts of F-Detector™ solution.

Fig 11. Signal Density According to the Amounts of F-Detector™ Solution

- **Plasmid DNA kit : DNA-spin™ kit**
- **Plasmid DNA : pD2HM-EGFP**
- **Host *E. coli* : JM110 (*endA*⁺ strain)**
- **$OD_{260/280}$: 1.81 ~ 1.83**
- **Adjust to 200ng/㎕ (EGFP)**
- **2㎕ loading at 1.0% Agarose gel**





- **Genelator™ : 11.2㎕ (15㎕ rxn.)**
- **EGFP plasmid DNA 2㎕**
- **F-Detector™ : 0.3㎕ ~ 2.0㎕**
- **1 hr *in vitro* reaction at 37℃**
- **12% SDS-PAGE**
- **Laser fluorescence scanner**

EGFP
29.6 KDa

Protocol : F-Detector™ Cell Free Labeling Preocedure

PROCEDURE (Non-Radioisotope Labeling Reaction)

1. Remove the tube containing the fluorescent modified tRNA solution from the freezer and allow it to thaw by warming by hand. After thawing, briefly centrifuge the tube for 2-3 seconds; immediately upon thawing place the tube on ice. If required, allow the tube in ice bath to cool to 2-4℃.

[NOTE] Always avoid multiple freeze-thaw cycles (do not subject to more than five freeze-thaw cycles) or exposure to frequent temperature changes. Dispense the product into smaller aliquots as needed.

2. Add 1㎕ F-Detector™ Solution per 10㎕ cell-free reaction volume (Table 4).

[NOTE] Appropriate volume of F-Detector™ tRNA solution is added to cell-free protein synthesis reaction mixture constituted with Genelator™ *in vitro* Transcription/ Translation Kit. For example, in order to carry out 15㎕-reaction, 0.5-2㎕ of fluorescent modified tRNA solution is added to reaction mixture. Generally 1㎕ addition per 15㎕-reaction is sufficient. The required volume of fluorescent modified tRNA solution is varied according to the expression level of target protein. For other reaction volumes, the volumetric portion of fluorescent modified tRNA solution should be maintained. Generally, as the volume of fluorescent modified tRNA solution is increased, the intensity of band corresponding to translated protein in gel becomes stronger.

3. Mix and centrifuge briefly to collect the reactions in the bottom of the tubes.

4. Incubate the reconstituted reaction mixture for an appropriate time (usually 60 min.) at 37℃ (Lower temperatures may be used for proteins that tend to aggregate).
(optional) To achieve optimal distribution of reaction components, samples should be shaken in a thermomixer or water-bath shaker.

5. Stop the reaction by placing on ice, and centrifuge briefly to collect the reactions in the bottom of the tubes.

6. Proceed with sample analysis such as SDS-PAGE or Functional Analysis.

The reaction mixture containing fluorescent labeled translated products Can be used in Gel Electrophoresis or Functional Analysis DIRECTLY.

Table 4. Pipetting Scheme for Co-Setup of F-Detector™ and Genelator™ Kits

Components	15㎕ Reaction Volume			45㎕ Reaction Volume		
	User Sample	Positive control	Negative control	User Sample	Positive control	Negative control
Genelator™ Master Mix	11.2㎕	11.2㎕	11.2㎕	33.6㎕	33.6㎕	33.6㎕
DNA Template	0.1㎍ ~ 0.5㎍	<2.0㎕	X	0.5㎍ ~ 2.0㎍	<5.0㎕	X
F-Detector™ Kit	1.0㎕	1.0㎕	1.0㎕	3.0㎕	3.0㎕	3.0㎕
RNase-Free Water	Add to 15㎕ volume	0.8㎕	2.8㎕	Add to 45㎕ volume	3.4㎕	8.4㎕

PROCEDURE (For SDS-PAGE Analysis)

This Gel Analysis Protocol is general iNtRON SDS-PAGE analysis method. If you have another protocol for SDS-PAGE analysis, there is no problem for analyzing protein.

1. When *in vitro* protein synthesis reaction is complete (or at any desired time point), remove 3~5㎕ aliquot. The remainder of the reaction may be stored at -20℃ or –70㎕ .

[NOTE] If you want to analyze proteins more than 10㎕ among above samples, we suggest precipitating the samples with acetone prior to the addition of SDS gel sample buffer (conventional acetone precipitation).

[OPTIONAL] Add RNase A to a final concentration of 0.2mg/ml to the sample and incubate at 37℃ for 10~30min to destroy the modified aminoacyl-tRNA. The presence of an 18KDa endogenous fluorescent band from fluorescent labeled aminoacyl-tRNA can be removed by treatment with RNase A. In the case that the molecular weight of target protein is not around 18KDa, this treatment is not required.

2. Add 20㎕ of 1㎕ SDS gel sample buffer to sample tube.

3. Thoroughly vortex for mixing and briefly centrifuge.

4. Heat for 2~5 min at 95℃ using heat block to denature the proteins.

[NOTE] Occasionally, denaturation at 100℃ results in the formation of large molecular weight species that do not migrate well on gels. Denaturing at 80~90℃ avoids formation of these large molecules. (**optional** : centrifuge at 13,000 rpm for 2~15 min without cooling to pellet the insoluble material.)

5. Carefully apply the supernatant of the denatured sample onto the gel (usually 12% SDS-PAGE or SDS-urea-PAGE gel).

6. Typically, electrophoresis is carried out at constant voltage of 50V in the stacking gel, and 100~150V in the separating gel. Electrophoresis is usually performed until Bromophenol Blue dye has run off the bottom of the gel.

7. Immediately after electrophoresis is completed, place the gel in water and then on a UV transilluminator in darkness, or in the laser-based fluorescent scanning instrument for analyzing proteins.

[NOTE] Fixing gel for a long period of time may decrease the signal intensity of the fluorescent-labeled *in vitro* translation products. The images of the fluorescent labeled proteins can be documented using equipment typically used to photograph Ethidium Bromide (EtBr)-stained DNA in agarose gels or captured using a digital photographic camera. For using a laser-based fluorescence scanning device, 490 nm wavelength is recommended as the best excitation source. If the band intensities appear to be somewhat weak in UV-based detection system due to low expression level, a laser-based fluorescence scanning may be used because it can increase the sensitivity at least 100-1000 fold compared to UV-based detection

PROCEDURE (For Functional Analysis of *in vitro* Synthesized Protein)

You can perform directly functional analysis of *in vitro* synthesized protein according to the characteristics of your protein.

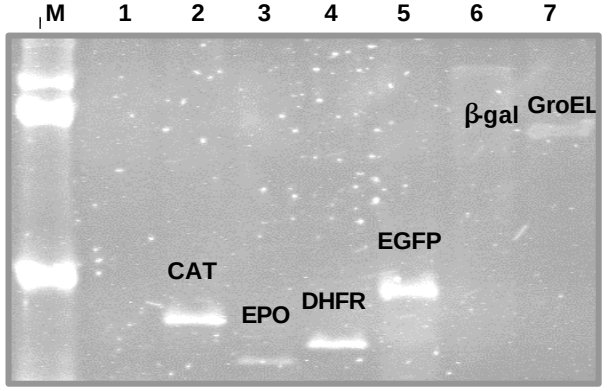
1. Centrifuge at 11,000 rpm for 10 min without cooling to obtain a soluble protein fraction (**optional**).

2. Use this supernatant directly in functional analysis such as *in vitro* bioassay or enzymatic activity assay.

Fig 12. Labeling and Detection of Various Protein Using F-Detector™ Kit
After SDS-PAGE, the gel analysis was performed with UV transilluminator (panel A) or Laser-based fluorescence scanner (panel B).
Various proteins were labeled with **F-Detector™ Cell Free Labeling Kit**. The 5μℓ of sample was treated with RNase to destroy the fluorescent labeled tRNA. The presence of an 18kDa endogenous fluorescent band from charged tRNA can be removed by treatment with RNase A. Under the optimal conditions, fluorescent bands from nanogram levels of *in vitro*-produced proteins could be detected directly from gels using a conventional UV-transilluminator. Higher sensitivity (~100-fold) can be obtained using a laser-based fluorescent gel scanner.
Lane 1 : Negative control (no DNA template), **lane 2 :** CAT plasmid DNA addition (25.6 KDa), **lane 3 :** EPO plasmid DNA addition (18.5 KDa), **lane 4 :** DHFR plasmid DNA addition (21.5 KDa), **lane 5 :** EGFP plasmid DNA addition (29.6 KDa), **lane 6 :** β-gal plasmid DNA addition (127 KDa), **lane 7 :** GroEL plasmid DNA addtion (57 KDa)

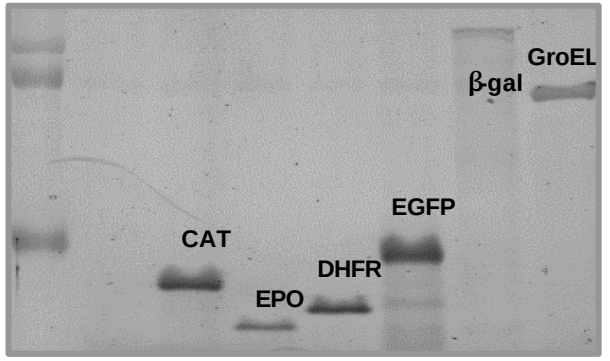
[PANEL A]

UV Transillumination



[PANEL B]

Laser-Based Fluorescent Scanning



Troubleshooting Guide

The Genelator™ and F-Detector™ Troubleshooting guide may be helpful in solving some problems that may occur. If you have other problems without this handbook, please contact our Technical Service Department.

Comments & Suggestions

No control protein visible

a) Nuclease contamination	Use RNase- and DNase-free tubes and tips.
b) Storage condition	The kits must be stored at -70℃ or -20℃.
c) Loss of activity	Check the expiration date, and storage condition

No target protein visible

a) Poor quality of DNA	Please use iNtRON DNA-spin™ plasmid DNA purification kit. Avoid adding excessive salts or glycerol when adding template DNA. The activity may be inhibited by NaCl (>50mM), glycerol (>1%), or by very small amounts of Mg^{2+}, or potassium salts.
b) Wrong quantity of DNA	Check the concentration and integrity of the DNA template. Titrate the amount of DNA used in the *in vitro* translation to determine the optimal amount.
c) RNase contamination	Omit RNase addition step in plasmid preparation
d) Error in promoter	Check the promoter of vector DNA. Make sure that the promoter is T7-based or Lac-based promoter.

Low expression yield

a) Nuclease contamination	Use RNase- and DNase-free tubes and tips.
b) Poor quality of DNA	Please use iNtRON DNA-spin™ plasmid DNA purification kit. Do not add RNase into Lysis solution. Use both Washing Buffer A and B.
c) Wrong quantity of DNA	Check the concentration and integrity of the DNA template. Add fully plasmid DNA without dH_2O into reaction tube.
d) Lac promoters	Add 1mM IPTG to translation reaction to overcome endogenous lac repressor.
e) RNase contamination	Omit RNase addition step in plasmid preparation

Low yield of active protein

a) Deficiency of post-translational modification	*E. coli* lysate can not introduce posttranslational modifications like glycosylation, phosphorylation, or signal-peptide cleavage.
b) Necessity of disulfide bond formation	Genelator™ contains reducing agents (equivalent to about 2mM DTT). For promotion of disulfide bond formation, dialysis of cell-free reaction sample to appropriate refolding buffer, or dilution of cell-free reaction sample with appropriate refolding buffer.

Comments & Suggestions

Protein insolubility

Protein aggregation	We recommended a 37℃ incubation for protein synthesis, but lower incubation temperatures may improve protein solubility.

Degradation of protein

a) Premature termination	Check the sequence of the target gene (open reading frame, mutation yielding a stop codon)
b) Protease contamination	Add EDTA-free protease inhibitors to the reaction.

No Fluorescence Signal

a) Error in protein synthesis	Check the expression of positive control DNA (EGFP).
b) Low F-Detector™ amount	Add more F-Detector™ in case of low expressed protein
c) Storage condition	The kits must be stored at -70℃ or -20℃.
d) Poor quality of DNA	Please use iNtRON DNA-spin™ plasmid DNA purification kit.
e) Wrong quantity of DNA	Check the concentration and integrity of the DNA template. Titrate the amount of DNA used in the *in vitro* translation to determine the optimal amount.
f) Lac promoter	Add 1mM IPTG to translation reaction to overcome endogenous lac repressor.
g) Premature termination	Check reading frame of DNA template (eg., stop codon mutation)
h) Protease contamination	Add EDTA-free protease inhibitors to the reaction.
i) Kit expired	Check the expiration date.
j) Weak fluorescent intensity	Leaving the fluorescent-labeled in vitro translation products alone for a long period (> 10 hrs) may decrease the signal intensity due to its hydrolysis. The labeled proteins prepared by F-Detector™ kit should be also handled according to the protocol.

Related Products

Products	Cat. No.	Size
PRO-PREP™ Protein Extraction Solution (For Cell/Tissue)	17081	100ml
PRO-MEASURE™ Protein Measurement Solution	21011	100ml
WEST-ZOL® (plus) Western Blot Detection System	16021	A sol. 100ml B sol. 100ml
PRO-STAIN™ Prestained Protein Marker I	24051	250μℓ
PRO-STAIN™ Prestained Protein Marker II	24061	250μℓ

SDS-PAGE Information

✍ Preparation of Resolving Gel (SDS-PAGE)

10% SDS-PAGE Gel	10ml	30ml	50ml
H_2O	3.97ml	11.9ml	19.83ml
1.5M Tris-HCl (pH 8.8)	2.5ml	7.5ml	12.5ml
30% Acryl/bis-	3.33ml	10ml	16.67ml
10% SDS	0.1ml	0.3ml	0.5ml
10% APS	0.1ml	0.3ml	0.5ml
TEMED	0.004ml	0.012ml	0.02ml

12% SDS-PAGE Gel	10ml	30ml	50ml
H_2O	3.3ml	9.9ml	16.5ml
1.5M Tris-HCl (pH 8.8)	2.5ml	7.5ml	12.5ml
30% Acryl/bis-	4ml	12ml	20ml
10% SDS	0.1ml	0.3ml	0.5ml
10% APS	0.1ml	0.3ml	0.5ml
TEMED	0.004ml	0.012ml	0.02ml

15% SDS-PAGE Gel	10ml	30ml	50ml
H_2O	2.3ml	6.9ml	11.5ml
1.5M Tris-HCl (pH 8.8)	2.5ml	7.5ml	12.5ml
30% Acryl/bis-	5ml	15ml	25ml
10% SDS	0.1ml	0.3ml	0.5ml
10% APS	0.1ml	0.3ml	0.5ml
TEMED	0.004ml	0.012ml	0.02ml

✍ Preparation of Stacking Gel (SDS-PAGE)

5% SDS-PAGE Gel	2ml	5ml	10ml
H_2O	1.337ml	3.442ml	6.885ml
1M Tris-HCl (pH 6.8)	0.25ml	0.625ml	1.25ml
30% Acryl/bis-	0.333ml	0.833ml	1.67ml
10% SDS	0.02ml	0.05ml	0.1ml
10% APS	0.02ml	0.05ml	0.1ml
TEMED	0.002ml	0.005ml	0.01ml

::: iNtRON Biotechnology, INC. :::

BULK ORDER (100 ea./one item order : 500 ea./one item order)
We are pleased to offer unpublished higher discounts on large volume purchases.

ITEM	CAT. NO.	SIZE
PRO-PREP™ Protein Extraction Kit	17081	100 ml
LPS Extraction Kit	17141	100 rxn.
i-StarMaster™ Master Mix PCR Kit	25241	96 tubes
DNA-spin™ Plasmid DNA Purification Kit	17093	250 col.
PCRquick-spin™ PCR Product Purification Kit	17202	250 col.
MEGA-spin™ Agarose Gel DNA Extraction Kit	17183	250 col.
RNA-spin™ Total RNA Extraction Kit	17211	50 col.
easy-BLUE™ Total RNA Extraction Solution	17061	100 ml
G-DEX™ IIb Genomic DNA Extraction Kit	17241	300T/200μℓ
PROBER™ Probe DNA Purifying System	17072	100 col.
WEST-ZOL® plus Western Blot Detection Kit	16021	200 ml

::: PRO-PREP™ Protein Extraction Kit CAT. 17081 Cells Tissues

Convenient Step !

Cells or Tissues Preparation
↓
Add PRO-PREP™ Solution
↓
Incubate on –20℃ for 10 min
↓
Spin Down

Just 10~15 min
No Protease inhibitor
Protease inhibitor Included

The iNtRON PRO-PREP™ Protein Extraction Kit is the first commercially available kit designed for efficient extraction of soluble proteins from cells or tissues. The PRO-PREP™ has been specifically formulated for utilization in Western blotting, immunoprecipitation, and kinase/phosphatase assays.

High Throughput & Mass Extraction

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