For research purpose only. Not for use in diagnostic procedures for clinical purposes. For IN VITRO USE ONLY.

easy-BLUE™ Total RNA Extraction Kit

Cat. No. 17061 100 ml

DESCRIPTION

There is about 1-2x10^{-5} μg of RNA per mammalian cell and we can theoretically obtain about 10-20μg of RNA by extracting 1x10^6 cells. easy-BLUE™ from iNtRON can makes RNA extraction rate nearing the theoretical number a reality. It has a clear advantage in dissolving RNA because it can obtain highly pure degree of RNA in contrast to the usual mal-dissolution problem due to a post-PPT contamination of protein, which is far too frequent in a RNA extraction.

KIT CONTENTS and STORAGE

One bottle containing 100ml in a box. Store in the dark at 4ºC.

PREPARING SOLUTION BEFORE USE

- Chloroform
- Isopropanol (2-propanol), Room temperature
- 70% EtOH, Room temperature
- Autoclaved or DEPC treated water for resolving total RNA
- P-Buffer (for Plant) : Final 0.8M sodium citrate
  1.2M NaCl

HOMOGENIZATION TECHNIQUES

1. *For Tissues* : Homogenize tissue samples in 1ml of easy-BLUE™ reagent per 50-100mg of tissue using a Homogenizer or equivalent. The sample volume should not exceed 10% of the volume of easy-BLUE™ reagent used for homogenization.
2. *For Cells (grown in monolayer)* : Lyse cells directly in a culture flask by adding 1ml of easy-BLUE™ reagent per 3.5cm diameter. An insufficient amount of reagent may result in contamination of the extracted total RNA with DNA and protein.
3. *For Cells (grown in suspension)* : Pellet cells by centrifugation. Lyse cells in this reagent by repetitive pipetting. Wahing cells before addition of easy-BLUE™ reagent should be avoided, because this increases the possibility of RNA degradation. Disruption of some yeast and bacterial cells may require the use of a homogenizer.

PRECAUTION FOR PREVENTING RNase

RNases can be introduced accidentally into the RNA extraction through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent in advance. Always wear disposable gloves. Also, use sterile, disposable plasticware and automatic pipettes reserved for RNA work to prevent cross-contamination with RNase from shared equipment.

PROTOCOL (For Cell)

1. Prepare 1-10x10^5 cell in 1.5ml tube. Centrifuge it to remove culture media (13,000rpm, 10sec), and add 1ml of easy-BLUE™.
   Note : In case of adherent cell, measure the viable count after trypsin treatment. In case of suspended cell, measure the viable count after centrifugation. Although 1ml of easy-BLUE™ is good for the preparation of up to 5-10x10^6 cell, it is recommended not to exceed 3-10x10^6 cell because RNA purity may fall with higher cell counts. Besides, in case of adherent cell, we can treat easy-BLUE™ in culture flask after removing culture medium, but doing so would waste a large amount of reagents and may result in the loss of harvested cell lysate. In any case, it is recommended to use after treatment of trypsin. Generally speaking, a T75 flask filled with adherent cells to about 75-80% volume would have 7-8X10^6 cell. In such case where an exact cell count is difficult to measure, use about 1/3 of volume and come up with an approximated cell count. However, it is always better to keep accurate cell count.
   For bacteria, Harvest 1-2ml of cells (OD_{600} : 0.8-1.0) by centrifuging at 13,000rpm for 1min. Remove supernatant.
2. Vigorously vortex in room temperature for 10sec.
   Note : This is actual cell lysis stage and is thus important to apply vortex until no clumps are seen. Once the cell is lysed, store it at 4℃. The sample is now stable at 4℃ up to a week.
3. Add 200㎕ of Chloroform and apply vortex.
   Note: Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper (blue layer) as the chloroform layer moves down. This region contains mixed parts of cell debris, protein, and genomic DNA and RNA. The purpose of adding the chloroform is to separate the phenol layer from aqueous layer and to eventually isolate RNA and genomic DNA/protein.
4. After centrifuging the solution at 13,000 rpm (4℃) for 10 min, transfer 400㎕ of the upper fluid to an empty 1.5ml tube.
   Note : Centrifugation of the solution creates two layers. The upper aqueous layer contains RNA while the lower phenol layer(blue color) contains denatured protein or cell debris etc. White sediments are formed at the boundary between two layers. This boundary layer contains mixtures of protein and genomic DNA. Protein and genomic DNA can be isolated from this boundary layer (Methods available upon request). When pipetting the upper layer, pay attention to to form any white sediments.
5. Add 400㎕ of isoprppanol (2-propanol) and mix it well by inverting the tube 2-3 times. Leave it for 10 min at room temperature.
   Note : This is a RNA dehydration stage. Use of EtOH is permitted, but in this case, one must use 800㎕ of EtOH. By adding isopropanol, one can observe the formation of a white layer, which contains RNA.
6. After centrifuging the solution at 13,000 rpm (4℃) for 5 min, remove the upper layer to obtain RNA pellet.
   Note : When the upper layer is discarded, white RNA pellets are left behind.
7. Add 1ml of 75% EtOH and mix the solution well by inverting the tube 2-3 times. Centrifuge the mixtures for 5 min at 10,000rpm (4℃). Discard the upper layer and dry the remaining RNA pellet.
   Note : This is a washing stage to remove impurities such as salts and etc. Once the mixture is centrifuged, RNA pellet turns white due to dehydration. When drying the RNA, carefully get rid of moisture on a tube wall with 3MM paper and dry the pellet for 5 min at RT. Please be careful not to over-dry the pellet, in which case it would be harder to dissolve RNA. Dapping the moisture on the tube wall with paper and 5 min of air-drying at room temperature would be enough.
8. Dissolve RNA using 20-50 ㎕ of DEPC treated distilled water.
   Note : It is important not to let the RNA pellet dry completely, because the over-dry will greatly decrease its solubility. Also, RNA can also be redissolved in 100% formamide (deionized) and stored at –70℃.

PROTOCOL (For Tissue)

1. Preparation of 50-100mg of fresh tissue.
2. Add 1ml of easy-BLUE™ reagent and homogenize tissue sample using a homogenizer or equivalent.
   Note : Homogenize tissue samples in 1ml of easy-BLUE™ reagent per 50-100mg of tissue using a homogenizer or equivalent. The sample volume should not exceed 10% of the volume of easy-BLUE™ reagent used for homogenization.
3. For preparation of RNA from tissue, follow step 2 of protocol (for cells).

PROTOCOL (For Plant)

1. Preparation of 10-100mg of plant sample.
   Note : Grind 10-100mg of samples to a powder in liquid nitrogen in a chilled mortar and pestle or commercial homogenizer.
2. Add 1ml of easy-BLUE™ reagent and homogenize plant sample using a homogenizer or equivalent.
   Note : Homogenize plant samples in 1ml of easy-BLUE™ reagent per 10-100mg of plant sample using a homogenizer or equivalent. The sample volume should not exceed 10% of the volume of easy-BLUE™ reagent used for homogenization.
3. For preparation of RNA from plant, follow step 2, 3, 4 of protocol (for cells).
4. Add 250㎕ of P-buffer(not provided) and 250㎕ of isoprppanol (2-propanol) per 1ml easy-BLUE™ reagent used for homogenization. Mx it well by inverting the tube 2-3 times leave it for 10 min at room temperature.
5. Follow step 6, 7, 8 of protocol (for cells).