INFUSION TECH

PURE™ Total RNA Extraction Kit

Cat No : PRG1010 / Package : 100 mL

RUO Only used for non-clinical purposes

DESCRIPTION

There are about 1-2×10^{-5} μg of RNA per mammalian cell and we can theoretically obtain about 10-20 μg of RNA by extracting 1×10^{6} cells. "PURE™ Total RNA Extraction Kit" from Infusion Tech can enable obtaining high amount of RNA near the theoretical value. As highly pure degree RNA are obtained, better results are observed in subsequent experiments.

KIT CONTENTS AND STORAGE

Single bottle containing 100 ml is packed in a box. Avoid direct light sources when storing at 4ºC.

MATERIALS TO BE SUPPLIED BY USER

1. Chloroform
2. Isopropanol (2-propanol), room temperature
3. 75% Ethanol, room temperature
4. Autoclaved or DEPC treated water for dissolving total RNA
5. Precipitation buffer : 0.8M Sodium citrate
   1.2M NaCl

HOMOGENIZATION TECHNIQUES

1. For Tissues : Homogenize tissue samples in 1 ml of PURE™ Total RNA Extraction Kit reagent per 10-100 mg of tissue using a homogenizer or a similar device. The sample volume should not exceed 10% of the volume of PURE™ Total RNA Extraction Kit reagent used for homogenization.

2. For Cells (grown in monolayer) : Lyse cells directly in a culture flask by adding 1 ml of PURE™ Total RNA Extraction Kit reagent per 3.5 cm 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 directly with PURE™ Total RNA Extraction Kit reagent by repetitive pipetting. Washing cells before addition of PURE™ Total RNA Extraction Kit 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 ACTIVITY

RNases can be introduced accidentally during the RNA extraction process due to 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 used for RNA work to prevent cross-contamination with RNase which potentially exist in equipments used by many users.

PROTOCOL

• For Cells

1. Prepare 1-10×10^{5} cells in 1.5 ml tube. Centrifuge to remove culture media (13,000 rpm, 10 sec), and add 1 ml of PURE™ Total RNA Extraction Kit reagent.
   → In case of adherent cells, measure the viable count after trypsin treatment. In case of suspended cells, measure the viable count after centrifugation. Although 1 ml of PURE™ Total RNA Extraction Kit reagent is good for the preparation of up to 5-10×10^{6} cells, it is recommended not to exceed 10×10^{6} cells because RNA purity may fall with higher cell counts. In case of, adherent cells, we can treat PURE™ Total RNA Extraction Kit reagent 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 7-8×10^{6} cells. In such case where an exact cell count is difficult to perform, use about 1/3 of volume and conduct an approximated cell count. However, it is always better to perform accurate cell count.
   → For bacteria, harvest 1-2 ml of cells (OD600 : 0.8-1.0) by centrifuging at 13,000 rpm for 1 min. Remove supernatant and then add 1 ml of PURE™ Total RNA Extraction Kit reagent.
2. Vigorously vortex in room temperature for 10 sec.
   → This is actual cell lysis stage and is thus important to perform vortexing until no clumps are seen. Once the cells are lysed, store at 4ºC. The sample is now stable at 4ºC up to a week.
3. Add 200 μl of chloroform and perform vortexing.
   → Observe if the mixture is cloudy overall. If not, perform additional vortexing. The purpose of adding chloroform is to separate the phenol layer from aqueous layer and to eventually isolate RNA.
4. After centrifuging the solution at 13,000 rpm (4ºC) for 10 min, transfer 400 μl of the upper fluid to an empty 1.5 ml tube.
   → Centrifugation of the solution creates 3 layers. The upper aqueous layer contains RNA while the lower phenol layer (blue color) contains denatured protein or cell debris etc. A white layer will also be observed just above the bottom blue layer as the chloroform layer moves down. This boundary layer contains mixtures of protein and genomic DNA.
5. Add 400 μl of isopropanol (2-propanol) and mix it well by inverting the tube 2-3 times. Leave it for 10 min at room temperature.
   → This stage is for RNA dehydration. Ethanol can also be used but as the efficiency is lower than isopropanol, 800 μl must be used. By adding ethanol or isopropanol, the formation of a white layer can be observed, which contains RNA.
6. After centrifuging the solution at 13,000 rpm (4ºC) for 5 min, remove the upper layer to obtain RNA pellet.
   → When the upper layer is discarded, white RNA pellets are left behind.
7. Add 1 ml of 75% ethanol and mix the solution well by inverting the tube 2-3 times. Centrifuge the mixture for 5 min at 10,000 rpm (4ºC). Discard the upper layer and dry the remaining RNA pellet.
   → This is the washing stage to remove impurities such as salts and etc. Once the mixture is centrifuged, the RNA pellet turns white due to dehydration. When drying the RNA, carefully get rid of moisture on the tube wall with 3MM paper and dry the pellet for 5 min at room temperature. Please be careful not to overdry the pellet, as in this case it would be harder to dissolve RNA.
   Carefully wiping the moisture on the tube wall with paper and 5 min of air-drying at room temperature is sufficient to prepare RNA.
8. Dissolve RNA using 20-50 μl of DEPC treated distilled water.
   → It is important not to let the RNA pellet dry completely, because overdry will greatly decrease its solubility. RNA can also be redissolved in 100% formamide (deionized) and stored at –70ºC.

• For Tissue

1. Prepare 10-100 mg of fresh tissue.
2. Add 1 ml of PURE™ Total RNA Extraction Kit reagent and homogenize tissue sample using a homogenizer or a similar device.
   → Homogenize tissue samples in 1 ml of PURE™ Total RNA Extraction Kit reagent per 10-100 mg of tissue using a homogenizer or a similar device. The sample volume should not exceed 10% of the volume of PURE™ Total RNA Extraction Kit reagent used for homogenization.
3. For preparation of RNA from tissue, follow step 2 of protocol (for cells).

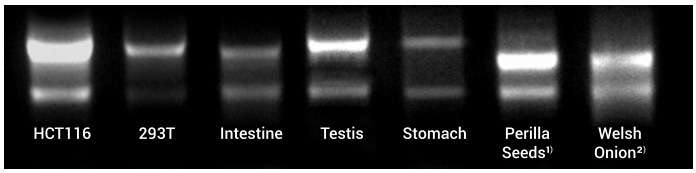
• For Plant

1. Prepare 10-100 mg of plant sample.
   → Grind 10-100 mg of samples to fine powder in liquid nitrogen in a chilled mortar and pestle or commercial homogenizer.
2. Add 1 ml of PURE™ Total RNA Extraction Kit reagent and homogenize the plant sample using a homogenizer or a similar device.
   → Homogenize plant samples in 1 ml of PURE™ Total RNA Extraction Kit reagent per 10-100 mg of plant sample using a homogenizer or a similar device. The sample volume should not exceed 10% of the volume of PURE™ Total RNA Extraction Kit reagent used for homogenization.
3. For preparation of RNA from plant, follow step 2, 3, 4 of protocol (for cells).
4. Add 250 µl of precipitation buffer (not provided) and 250 µl of isopropanol (2-propanol) per 1 ml PURE™ Total RNA Extraction Kit reagent used for homogenization. Mix it well by inverting the tube 2-3 times and incubate for 10 min at room temperature.
5. Follow step 6, 7, 8 of protocol (for cells).

EXPERIMENTAL INFORMATION

1. Total RNA Extraction from cells and tissues

Total RNA was isolated using PURE™ Total RNA Extraction Kit reagent and qualitatively analyzed by gel electrophoresis.



[1)] Extracted from the seeds of *Perilla frutescens var. japonica Hara*(들깨).
[2)] Extracted from the stalk of *Allium fistulosum*(대파).

Figure 1. Gel analysis of total RNA isolated from tissues and cells using PURE™ Total RNA Extraction Kit
Lane 1-2, cell line; **Lane 3-5**, mouse tissue; **Lane 6-7**, plant

2. Comparison of yield according to cell numbers

The PURE™ Total RNA Extraction Kit provides a simple and rapid method for the isolation of total RNA. Total RNA was isolated using PURE™ Total RNA Extraction Kit from varying amounts of cells and analyzed by gel electrophoresis.

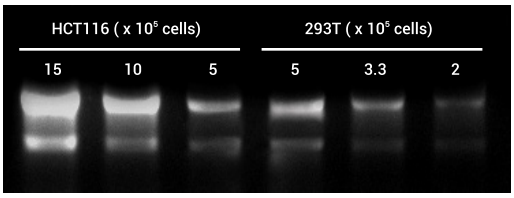


Figure 2. Gel analysis of total RNA isolated form HCT116 and 293T cells using PURE™ Total RNA Extraction Kit
The numbers on Figure 2 are the number of cells used.

3. RT-PCR amplification

Total RNA was purified from different tissues and cells using PURE™ Total RNA Extraction Kit. One step RT-PCR reaction was performed for β-actin (104 bp) gene from RNA and target gene expression was analyzed by gel electrophoresis.

Figure 3. RT-PCR amplification for β-actin
Lane M, 100bp ladder DNA marker; **Lane 1**, HCT116 cells;
Lane 2, 293T cells; **Lane 3**, mouse intestine; **Lane 4**, mouse spleen

4. Comparison of total RNA yield from different company kits

In order to compare the total RNA yield, the absorbance of RNA samples extracted using PURE™ Total RNA Extraction Kit and two other suppliers were measured by spectrophotometric analysis.

Sample	Product	*Concentration (ng/µl)	A_{260} / A_{280}	Total amount (µg)
HCT116 (1 x 10^6 cells)	Supplier A	542.66	2.02	27.13
	Supplier B	506.90	2.04	25.35
	PURE™ Total RNA Extraction Kit	662.33	2.03	33.12
Muscle (50 mg)	Supplier A	142.34	1.92	7.12
	Supplier B	108.03	1.91	5.40
	PURE™ Total RNA Extraction Kit	196.26	1.93	9.81

* Elution volume used in this experiment was 50 µl

TROUBLESHOOTING GUIDE

Problem	Possible Cause	Recommendation
Low RNA yield	Incomplete lysis	· Incomplete homogenization or lysis of sample. · Final RNA pellet incompletely redissolved.
A_{260}/A_{280} < 1.65		· The aqueous phase was mixed and contaminated with the phenol phase. · Do not transfer more than 400 µl at step 4 of protocol (for cells). · The amount of reagent used was too small compared to sample amount on step 4 of protocol (for cells)
RNA degradation	Sample condition	· Tissues were not immediately processed or frozen after acquired. · Aqueous solutions or tubes were contaminated and not RNase free.

RELATED PRODUCTS

Product Name	Cat. No.
PURE™ Strong Plant RNA Extraction Kit	PRG821
PURE™ Strong Tissue RNA Extraction Kit	PRG621
PURE™ Plant RNA Extraction Kit	PRG811
PURE™ Cell&Tissue RNA Extraction Kit	PRG611
PURE™ Viral RNA Extraction Kit	PRG111
Water, DEPC Treated Solution, 500 ml	IFT-1003
Water, DEPC Treated Solution, 1000 ml	IFT-1002
Water, DEPC Treated Solution, 10 L	IFT-1001

Symbols

Symbols	Description
RUO	Research use only
REF	Catalog number
LOT	Batch code
	Use by YYYY-MM-DD

GHS-Labeling

INFUSION TECH
#1202, 12F, 8, Heungan-daero 427beon-gil, Dongan-gu, Anyang-si, Gyeonggi-do, Korea
TEL +82-031-687-3459 FAX +82-031-624-1518 https://infusiontech.co.kr