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

ISO 9001/14001 Certified Company

easy-RED™ Total RNA Extraction Kit [for liquid sample]

Cat. No. 17063 100 ml

DESCRIPTION

easy-RED™ Total RNA Extraction Kit for liquid sample is a complete, ready to use reagent for easy isolation of total RNA from liquid samples. easy-RED™ Kit is similar to the original easy-BLUE™ Kit in composition and result of use. However, easy-RED™ Kit is designed for use with liquid sample as culture fluid, serum and virus preparations in which large volumes of sample need to be processed while easy-BLUE™ Kit is designed for cell suspension or tissues. It is formulated to accommodate processing of more liquid sample per unit of reagent compared to the original formula. The only difference between easy-BLUE™ Kit and easy-RED™ Kit is slightly more concentrated formula to allow lower quantities of reagent to be used relative to sample (easy-BLUE™ Solution = 10:1 required, easy-RED™ Solution = 3:1 required). The two reagent can be distinguished by color (easy-RED™ Solution = deep-pink red, easy-BLUE™ Solution = dark-cyan blue).

easy-RED™ Kit facilitates isolation of a variety of RNA species of large or small molecular size. For example, RNA isolated from mouse liver, electrophoresed on an agarose gel, and stained with ethidium bromide, shows discrete bands of high molecular weight RNA between 7 kb and 15 kb in size, two predominant ribosomal RNA bands at ~5 kb and at ~2 kb, and low molecular weight RNA between 0.1 and 0.3 kb (tRNA, 5S). The isolated RNA has an A_{260}/A_{280} ratio ≥ 1.8 diluted into TE.

KIT CONTENTS and STORAGE

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

PREPARING SOLUTION BEFORE USE

Chloroform
Isopropanol (2-propanol), Room temperature
70% ethanol (in RNase-free water), Room temperature
P-buffer (for plant or stool) : Final 0.8M sodium citrate / 1.2M NaCl
RNase-free water, RNase-free PBS
[To prepare RNase-free water, draw water into RNase-free glass bottles. Add diethylpyrocarbonate (DEPC) to 0.01% (v/v). Let stand overnight and autoclave.]

PRECAUTION FOR PREVENTING RNase CONTAMINATION

RNases can be introduce accidentally into the RNA preparation at any point in the isolation procedure through improper technique. Because RNase activity is difficult to inhibit, it is essential to prevent its introduction. The following guidelines should be observed when working the RNA.

- Always wear disposable gloves. Skin often contain bacteria and molds that can contaminate an RNA preparation and be a source of RNases. Practice good microbial technique to prevent microbial contamination.
- Use sterile, disposable plasticware and automatic pipette reserved for RNA work to cross-contamination with RNases from shared equipment.
- In the presence of easy-RED™, RNA is protected from RNase contamination. Downstream sample handling requires that nondisposable glassware or plasticware be RNase-free. Glass items can be baked at 150℃ for 4 hours, and plastic items can be soaked for 10 minutes in 0.5 M NaOH, rinse thoroughly with water, and autoclaved.

OTHER PRECAUTION

- Use of disposable tubes made of clear polypropylene is recommended when working with less than 2 ml volumes of easy-RED™ Solution.
- For larger volumes, use glass or polypropylene tubes, and test to be sure that the tubes can withstand 12,000 x g with easy-RED™ Solution and chloroform. Do not use tubes that leak or crack.
- Carefully equilibrate the weights of the tubes prior to centrifugation
- Glass tubes must be sealed with parafilm topped with a layer of foil, and polypropylene tubes must be capped before centrifugaton.

Cautions : When working with easy-RED™ Solution use gloves and eye protection (shield, safty goggles). Avoid contact with skin or clothing. Use in a chemical fume hood. Avoid breathing vapor.

PROTOCOL (for Liquid Sample - Blood, Serum, Biological Fluid)

1. Preparation 250µl of sample in 1.5 ml microcentrifuge tube and add 750µl of easy-RED™ Solution.
   Note : *For Biological Fluids* - For each 250µl of sample add 750µl of easy-RED™.Solution. Lyse cells in the sample suspension by passing the suspension several times through a pipette. Use at least 750µl of easy-RED™ Solution per 5 ~ 10 x 10^6 cells. Biological fluids such as whole blood which contain high level of contaminating material may be may be diluted 1 : 1 with RNase free water. The volume ratio of easy-RED™ Solution to sample should always be 3:1.
2. Mix the sample in room temperature for 15 sec by vigorously vortex and incubate the tube at room temperature (15 ~ 30℃) for 5 min.
   Note : This is actual sample lysis stage and is thus important to apply vortex until no clumps are seen. Once the cell lysed, store it at 4℃. The sample is now stable at 4℃ up to a week.
   Note : An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material. Following homogenization, remove insoluble material from homogenate by centrifugation at 13,000 rpm for 10 min at 4℃. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while supernatant contains RNA. In sample from fat tissue, an excess of fat collects as top layer which should be removed. In each case, transfer the cleared homogenate solution to a clean tube and proceed with chloroform addition and phase separation as described.
3. Add 200µl of Chloroform and mix the sample in room temperature for 15 sec by vigorously vortex. Then incubate the tube at room temperature for 5 min.
   Note : Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper 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 eventually to isolate RNA and genomic DNA/protein.
4. After centrifuging the tube at 13,000 rpm (4℃) for 15 min, transfer 400µl of the upper fluid to a new 1.5 ml centrifuge tube.
   Note : Centrifugation of the solution creates two layers. The upper aqueous layer contains RNA while the lower phenol layer (red color) contains denatured protein or cell debris etc. White sediment s 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 form this boundary layer (Method available upon request). When pipetting the upper layer, pay attention to form any white sediments .
5. Add equal volume (400µl) of isopropanol (2-propanol) and mix it well by inverting the tube 4 ~ 5 times. Incubate the tube for 10 min at room temperature.
   Note : This is RNA precipitation stage. Use of ethanol is permitted, but in this case, it must use 800µl (2 volumes) of ice-cold ethanol and store for 30 min at -20℃. By adding isopropanol, one can observe the formation of a white layer, which contains RNA.
6. After centrifuging the tube at 13,000 rpm (4℃) for 10 min, carefully remove the supernatant without disturbing the pellet.
   Note : When the upper layer is discarded, white RNA pellets are left bottom of the tube.
   Note : Table-top centrifuges that can attain a maximum of 2,600 x g are suitable for use in these protocols if the centrifugation time is increased to 30 ~ 60 min in step 4, 6.
7. Add 1 ml of 70% ethanol and mix the solution well by inverting the tube 4 ~ 5 times. Centrifuge the mixture for 5 min at 13,000 rpm (4℃). Carefully discard the supernatant without disturbing the pellet. Then dry the remaining RNA pellet.
   Note : This is a washing step to remove impurities such as salt and etc. Once the mixture is centrifuged, RNA pellet turns white due to dehydration. When drying tube RNA, carefully get rid of moisture on a tube wall with 3MM paper and dry the pellet for 5 min at RT. Do not dry the pellet by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA sample have an A260/A280 ratio < 1.6.
8. Dissolve RNA using 20 - 50 ml of RNase free water.
   Note : Dissolve RNA in RNase free water or 0.5% SDS solution by passing the solution a few times through a pipette tip. 0.5 % SDS should not be used if RNA will undergo further enzymatic reaction. RNA can also be in 100% formamide (deionized) and store at -70℃.

PROTOCOL (for Cultured Cell, Gram-Negative Bacteria)

1. Preparation sample in 1.5 ml microcentrifuge tube and add 750µl of easy-RED™ Solution.

   Note 1 : *For Cells Grown in Monolayer* - Lyse cells directly in a culture dish by adding 300 ~ 400µl of easy-RED™ Solution to a 3.5 cm diameter dish, and passing the cell lysate, and passing the cell lysate several times through a pipette. The amount of easy-RED™ Solution is based on the area of the culture dish (0.3 ~ 0.4 ml per 10 cm^2) and not on the number of cell present. Do not add water to the homogenate. The volume of the medium adhering to the culture dish dilutes the homogenate sufficiently.

   Note 2 : *For Cells Grown in Suspension* - Pellet cell by centrifugation. Resuspend the appropriate number of cell (animal cell - 1 ~ 3 x 10^6, Gram negative bacteria - 1 x 10^7) into 250µl of medium or PBS thoroughly by pipetting, tapping or vortexing. Add 750µl of easy-RED™ Solution into cell suspension then vortex the tube vigorously.
2. Mix the sample in room temperature for 15 sec by vigorously vortex and incubate the tube at room temperature for 5 min.

   Note : This is actual sample lysis stage and is thus important to apply vortex until no clumps are seen. Once the cell lysed, store it at 4℃. The sample is now stable at 4℃ up to a week.
3. Add 200µl of Chloroform and mix the sample in room temperature for 15 sec by vigorously vortex. Then incubate the tube at room temperature for 5 min.

   Note : Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper 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 eventually to isolate RNA and genomic DNA/protein.
4. After centrifuging the tube at 13,000 rpm (4℃) for 15 min, transfer 400µl of the upper fluid to a new 1.5 ml centrifuge tube.

   Note : Centrifugation of the solution creates two layers. The upper aqueous layer contains RNA while the lower phenol layer (red color) contains denatured protein or cell debris etc. White sediment s 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 form this boundary layer (Method available upon request). When pipetting the upper layer, pay attention to form any white sediments .
5. Add equal volume (400µl) of isopropanol (2-propanol) and mix it well by inverting the tube 4 ~ 5 times. Incubate the tube for 10 min at room temperature.

   Note : This is RNA precipitation stage. Use of ethanol is permitted, but in this case, it must use 800µl (2 volumes) of ice-cold ethanol and store for 30 min at -20℃. By adding isopropanol, one can observe the formation of a white layer, which contains RNA.
6. After centrifuging the tube at 13,000 rpm (4℃) for 10 min, carefully remove the supernatant without disturbing the pellet.

   Note : When the upper layer is discarded, white RNA pellets are left bottom of the tube.

   Note : Table-top centrifuges that can attain a maximum of 2,600 x g are suitable for use in these protocols if the centrifugation time is increased to 30 ~ 60 min in step 4, 6.
7. Add 1 ml of 70% ethanol and mix the solution well by inverting the tube 4 ~ 5 times.
   Centrifuge the mixture for 5 min at 13,000 rpm (4℃). Carefully discard the supernatant without disturbing the pellet. Then dry the remaining RNA pellet.

   Note : This is a washing step to remove impurities such as salt and etc. Once the mixture is centrifuged, RNA pellet turns white due to dehydration. When drying tube RNA, carefully get rid of moisture on a tube wall with 3MM paper and dry the pellet for 5 min at RT. Do not dry the pellet by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA sample have an A_{260}/A_{280} ratio < 1.6.
8. Dissolve RNA using 20 - 50 ml of RNase free water.

   Note : Dissolve RNA in RNase free water or 0.5% SDS solution by passing the solution a few times through a pipette tip. 0.5 % SDS should not be used if RNA will undergo further enzymatic reaction. RNA can also be in 100% formamide (deionized) and store at -70℃.

PROTOCOL (for Animal Tissue)

1. Preparation sample in 1.5 ml microcentrifuge tube and add 750µl of easy-RED™ Solution.

   Note : When disrupt the tissue samples with mortar under liquid nitrogen, add 750µl of easy-RED™ Solution per 10 ~ 100 mg of tissue. When disrupt tissue samples with micro-pestle or mechanical tissue grinder under room temperature, prepare the ground tissue suspension using 250µl of PBS (DEPC treated) or DDW per 10 ~ 100 mg of tissue sample. If tissue sample volume is < 250µl, adjust the volume to 250 µl with RNase free water
2. Mix the sample in room temperature for 15 sec by vigorously vortex and incubate the tube at room temperature for 5 min.

   Note : This is actual sample lysis stage and is thus important to apply vortex until no clumps are seen. Once the cell lysed, store it at 4℃. The sample is now stable at 4℃ up to a week.

   Note : An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material. Following homogenization, remove insoluble material from homogenate by centrifugation at 13,000 rpm for 10 min at 4℃. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while supernatant contains RNA. In sample from fat tissue, an excess of fat collects as top layer which should be removed. In each case, transfer the cleared homogenate solution to a clean tube and proceed with chloroform addition and phase separation as described.
3. Add 200µl of Chloroform and mix the sample in room temperature for 15 sec by vigorously vortex. Then incubate the tube at room temperature for 5 min.

   Note : Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper 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 eventually to isolate RNA and genomic DNA/protein.
4. After centrifuging the tube at 13,000 rpm (4℃) for 15 min, transfer 400µl of the upper fluid to a new 1.5 ml centrifuge tube.

   Note : Centrifugation of the solution creates two layers. The upper aqueous layer contains RNA while the lower phenol layer (red color) contains denatured protein or cell debris etc. White sediment s 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 form this boundary layer (Method available upon request). When pipetting the upper layer, pay attention to form any white sediments .
5. Add equal volume (400µl) of isopropanol (2-propanol) and mix it well by inverting the tube 4 ~ 5 times. Incubate the tube for 10 min at room temperature.

   Note : This is RNA precipitation stage. Use of ethanol is permitted, but in this case, it must use 800µl (2 volumes) of ice-cold ethanol and store for 30 min at -20℃. By adding isopropanol, one can observe the formation of a white layer, which contains RNA.
6. After centrifuging the tube at 13,000 rpm (4℃) for 10 min, carefully remove the supernatant without disturbing the pellet.

   Note : When the upper layer is discarded, white RNA pellets are left bottom of the tube.

   Note : Table-top centrifuges that can attain a maximum of 2,600 x g are suitable for use in these protocols if the centrifugation time is increased to 30 ~ 60 min in step 4, 6.
7. Add 1 ml of 70% ethanol and mix the solution well by inverting the tube 4 ~ 5 times.
   Centrifuge the mixture for 5 min at 13,000 rpm (4℃). Carefully discard the supernatant without disturbing the pellet. Then dry the remaining RNA pellet.

   Note : This is a washing step to remove impurities such as salt and etc. Once the mixture is centrifuged, RNA pellet turns white due to dehydration. When drying tube RNA, carefully get rid of moisture on a tube wall with 3MM paper and dry the pellet for 5 min at RT. Do not dry the pellet by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA sample have an A_{260}/A_{280} ratio < 1.6.
8. Dissolve RNA using 20 – 50µl of RNase free water.

   Note : Dissolve RNA in RNase free water or 0.5% SDS solution by passing the solution a few times through a pipette tip. 0.5 % SDS should not be used if RNA will undergo further enzymatic reaction. RNA can also be in 100% formamide (deionized) and store at -70℃.

PROTOCOL (for Stool)

1. Preparation of 100 ~ 200 mg of stool sample add 200µl of PBS then mix thoroughly by vigorous vortexing.
   Note : The moist and fresh stool sample is good to RNA isolation. If the sample is frozen, use a scalpel or spatula to scrape bits of stool into a 2 ml microcentrifuge tube on ice. When using frozen stool samples, take care that the samples do not thaw until PBS is added to resuspend the sample; otherwise the RNA in the sample may degrade. After addition of easy-RED™ Solution all following steps can be performed at room temperature. If the sample is a liquid form, pipette 200µl into the microcentrifuge tube. Cut the end of the pipette tip to make pipetting easier.
2. Add 750µl of easy-RED™ Solution and vigorously vortex for 15 sec. Incubate the mixture for 5 min at room temperature.
   Note : An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material. Following homogenization, remove insoluble material from homogenate by centrifugation at 13,000 rpm for 10 min at 4℃. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while supernatant contains RNA. In sample from fat tissue, an excess of fat collects as top layer which should be removed. In each case, transfer the cleared homogenate solution to a clean tube and proceed with chloroform addition and phase separation as described.
3. Add 200µl of Chloroform and mix the sample in room temperature for 15 sec by vigorously vortex. Then incubate the tube at room temperature for 5 min.
   Note : Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper 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 eventually to isolate RNA and genomic DNA/protein.
4. After centrifuging the tube at 13,000 rpm (4℃) for 15 min, transfer 400µl of the upper fluid to a new 1.5 ml centrifuge tube.
5. Add 250µl of P-buffer (not provided) and 250µl of isopropanol. Mix it well by inverting the tube 5 ~ 6 times leave it for 10 min at room temperature.
6. After centrifuging the tube at 13,000 rpm (4℃) for 10 min, carefully remove the supernatant without disturbing the pellet.
   Note : When the upper layer is discarded, white RNA pellets are left bottom of the tube.
7. Add 1 ml of 70% ethanol and mix the solution well by inverting the tube 4 ~ 5 times. Centrifuge the mixture for 5 min at 13,000 rpm (4℃). Carefully discard the supernatant without disturbing the pellet. Then dry the remaining RNA pellet.
   Note : This is a washing step to remove impurities such as salt and etc. Once the mixture is centrifuged, RNA pellet turns white due to dehydration. When drying tube RNA, carefully get rid of moisture on a tube wall with 3MM paper and dry the pellet for 5 min at RT. Do not dry the pellet by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA sample have an A_{260}/A_{280} ratio < 1.6.
8. Dissolve RNA using 20 – 50µl of RNase free water.
   Note : Dissolve RNA in RNase free water or 0.5% SDS solution by passing the solution a few times through a pipette tip. 0.5 % SDS should not be used if RNA will undergo further enzymatic reaction. RNA can also be in 100% formamide (deionized) and store at -70℃.

PROTOCOL (for Plant)

1. Preparation of 10 ~ 100 mg of plant sample in 1.5 ml microcentrifuge tube and add 750µl of easy-RED™ Solution
   Note : When grind the plant samples with mortar under liquid nitrogen, add 750 µl of easy-RED™ Solution per 10 - 100 mg of plant tissue. When disrupt plant tissue samples with micro-pestle or mechanical tissue grinder under room temperature, prepare the ground tissue suspension using 250µl of PBS (DEPC treated) or DDW per 10 - 100 mg of plant tissue sample. If plant tissue sample volume is < 250µl, adjust the volume to 250µl with RNase free water. Then lyse the sample with 750µl of easy-RED™ Solution.
2. Mix the sample in room temperature for 15 sec by vigorously vortex and incubate the tube at room temperature for 5 min.
   Note : An additional isolation step may be required for samples with high content of proteins, fat, polysaccharides or extracellular material. Following homogenization, remove insoluble material from homogenate by centrifugation at 13,000 rpm for 10 min at 4℃. The resulting pellet contains extracellular membranes, polysaccharides, and high molecular weight DNA, while supernatant contains RNA. In sample from fat tissue, an excess of fat collects as top layer which should be removed. In each case, transfer the cleared homogenate solution to a clean tube and proceed with chloroform addition and phase separation as described.
3. Add 200µl of Chloroform and mix the sample in room temperature for 15 sec by vigorously vortex. Then incubate the tube at room temperature for 5 min.
   Note : Observe the tube before vortexing. When Chloroform is added, one would see a white line being formed just beneath the upper 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 eventually to isolate RNA and genomic DNA/protein.
4. After centrifuging the tube at 13,000 rpm (4℃) for 15 min, transfer 400µl of the upper fluid to a new 1.5 ml centrifuge tube.
5. Add 250µl of P-buffer (not provided) and 250µl of isopropanol. Mix it well by inverting the tube 5 ~ 6 times leave it for 10 min at room temperature.
6. After centrifuging the tube at 13,000 rpm (4℃) for 10 min, carefully remove the supernatant without disturbing the pellet.
   Note : When the upper layer is discarded, white RNA pellets are left bottom of the tube.
7. Add 1 ml of 70% ethanol and mix the solution well by inverting the tube 4 ~ 5 times. Centrifuge the mixture for 5 min at 13,000 rpm (4℃). Carefully discard the supernatant without disturbing the pellet. Then dry the remaining RNA pellet.
   Note : This is a washing step to remove impurities such as salt and etc. Once the mixture is centrifuged, RNA pellet turns white due to dehydration. When drying tube RNA, carefully get rid of moisture on a tube wall with 3MM paper and dry the pellet for 5 min at RT. Do not dry the pellet by centrifugation under vacuum. It is important not to let the RNA pellet dry completely as this will greatly decrease its solubility. Partially dissolved RNA sample have an A260/A280 ratio < 1.6.
8. Dissolve RNA using 20 – 50µl of RNase free water.
   Note : Dissolve RNA in RNase free water or 0.5% SDS solution by passing the solution a few times through a pipette tip. 0.5 % SDS should not be used if RNA will undergo further enzymatic reaction. RNA can also be in 100% formamide (deionized) and store at -70℃.

EXPERIMENTAL INFORMATION

● *Total RNA preparation from different kinds of samples*

The easy-RED™ Total RNA Extraction Kit provides a simple and rapid method for the isolation of total RNA from various samples (eg. cultured cell, bacteria, blood, swab, stool, plant, viral origin etc.)

Samples	Start mounts	Yield (mg)	$A_{260/280}$
Human Whole Blood	250µl	4 ~ 9	1.82
Stool	200 mg	5 ~ 10	1.78
Cell Swab	1 ea	7 ~ 15	1.80
Buccal Swab	1 ea	ND *	ND *
Virus Fluid	250µl	ND *	ND *
SNU-1	1 x 10^6 cells	15 ~ 25	1.89
K-562	1 x 10^6 cells	15 ~ 25	1.88
Liver (Mouse)	25 mg	190 ~ 220	1.87
Heart (Mouse)	25 mg	150 ~ 180	1.88
Brain (Mouse)	25 mg	150 ~ 180	1.86
Kidney (Mouse)	25 mg	200 ~ 220	1.85
E. coli	OD_{600} = 1.0 x 2 ml	10 ~ 20	1.89
Green pepper	50 mg	12 ~ 24	1.77
Pea (Seed)	10 mg	10 ~ 20	1.72

* ND means 'not detectable'

TECHNICAL INFORMATION

• *Comparison of yield according to sample volume*

The easy-RED™ Total RNA Extraction Kit was designed to show strong and stable RNA extraction from liquid samples.

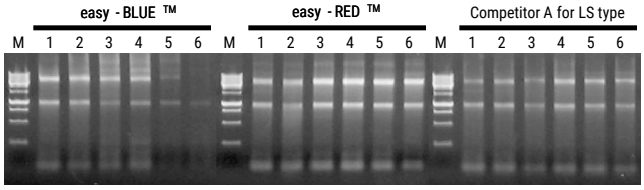

Fig. 1. Gel analysis of total RNA isolated from different volume of sample (condition under equal number of cell) using easy-BLUE™ Total RNA Extraction Kit, easy-RED™ Total RNA Extraction Kit and Competitor A product for liquid sample.

Lane M, 1kb Ladder DNA Marker; **lane 1**, 50µl of sample; **lane 2**, 100µl of sample; **lane 3**, 250µl l of sample; **lane 4**, 300µl of sample; **lane 5**, 400µl of sample ; **lane 6**, 500 µl of sample

• *Comparison of yield according to clinical samples*

The easy-RED™ Total RNA Extraction Kit shows considerable efficiency of RNA extraction from clinical samples (eg. blood, stool, swab etc)

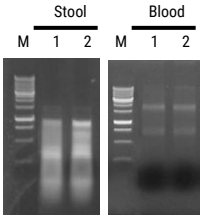

Fig. 2. Gel analysis of total RNA isolated from stool and whole blood (human) using easy-RED™ Total RNA Extraction Kit and Competitor A product for liquid sample.

Lane M, 1kb Ladder DNA Marker; **lane 1**, extracted RNA using Competitor A product for liquid sample; **lane 2**, extracted RNA using easy-RED™ Total RNA extraction

• *Comparison of purifying efficiency according to human blood samples*

In order to isolate intact RNA from whole blood using easy-RED™ Total RNA Extraction Kit, blood sample be may be diluted 1 : 1 with RNase free water. easy-RED™ Total RNA Extraction Kit shows an improved activity of gDNA elimination better than Competitor A product.

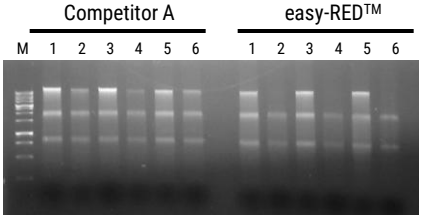

Fig. 3. Gel analysis of total RNA isolated from stool and whole blood (human) using easy-RED™ Total RNA Extraction Kit and Competitor A product for liquid sample.

Lane M, 1kb Ladder DNA Marker; **lane 1**, undiluted blood w/o anticoagulation reagent; **lane 2,** 1/2 diluted blood w/o anticoagulation reagent; **lane 3,** undiluted blood treat with EDTA; **lane 4,** 1/2 diluted blood treat with EDTA; **lane 5,** undiluted blood treat with heparin; **lane 6,** 1/2 diluted blood treat with heparin.

• *RT-PCR amplification*

The easy-RED™ Total RNA Extraction Kit shows advanced efficiency of viral RNA extraction from biological fluid. Improved efficiency was verified RT-PCR sensitivities.

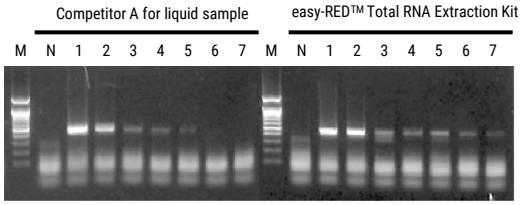

Fig. 4. Viral RNA isolation and RT-PCR amplification

In order to estimate efficiency of viral RNA extraction, The viral RNAs which isolated using different RNA extraction Kits were diluted serially then conserved viral gene was amplified using *Maxime*™ RT-PCR PreMix (Cat. No. 25131).

Lane M, 100bp Ladder DNA Marker; **lane N**, Negative control (No template); **lane 1**, Undiluted viral RNA ($10^{5.0}$EID50/0.1ml of sample); **lane 2**, 10^{-1} diluted; **lane 3**, 10^{-2} diluted; **lane 4**, 10^{-3} diluted; **lane 5**, 10^{-4} diluted; **lane 6**, 10^{-5} diluted; **lane 7**, 10^{-6} diluted.

TROUBLESHOOTING GUIDE

Problem	Possible Cause / Recommendation
Low RNA Yield	- Incomplete homogenization or lysis of sample. - Final RNA pellet incompletely dissolved.
A_{260}/A_{280} < 1.6	- The aqueous phase was contaminated with the phenol phase. - Sample homogenized in too small a reagent volume. - Incomplete dissolution of the final RNA pellet.
RNA degradation	- Sample was preserved in bad-condition. - Cells were dispersed by trypsin digestion. - Aqueous solutions or tubes were not RNAse free.
gDNA carry-over	- Sample homogenized in too small a reagent volume. - Sample used for the isolation contained organic solvent (e.g., DMSO, ethanol etc.), Strong buffer, or alkaline solution.
Proteoglycan and polysaccharide contamination	- The following modification of the RNA precipitation step removes these contaminant from the isolated RNA. Add to the aqueous 250 ml of isopropanol followed by 250 ml of P-buffer (0.8 M sodium citrate and 1.2 M NaCl) per 400 ml of aqueous phase. Mix the resulting solution, centrifuge and proceed with the isolation as described in the protocol.

RELATED PRODUCTS

Product Name	Cat. No.
easy-BLUE™ Total RNA Extraction Kit	17061
easy-spin™ Total RNA Extraction Kit	17221
ONE-STEP RT-PCR PreMix Kit	25101
Maxime™ RT PreMIx (Random)	25082
Maxime™ RT PreMix (Oligo dT)	25081
Maxime™ RT-PCR PreMix	25131
Maxime™ PCR PreMix (i-StarTaq)	25165
SiZer™-100 DNA Marker Solution	24073
SiZer™-1000 DNA Marker Solution	24074
SiZer™-1000 plus DNA Marker Solution	24075