TripleXtractor dirctRNA Kit

#GK23.0100 (100 preps) | GK23s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : Up to 200µl of body fluids (whole blood, buffy coat, serum, etc.) up to $5x10^6$ cultured animal yeast or plant cells, 10-100 mg of tissue (animal or plant), up to $1x10^9$ bacterial cells

Expected Yield : total RNA (e.g., up to 3 µg from 200 µl of whole human blood, up to 20 µg from $1x10^9$ cells of *Escherichia coli*)

Format : spin column

Operation Time : within 15 minutes

Elution Volume : 25-100µl

Product: The tripleXtractor directRNA kit combines the strong lysis capability of the monophasic phenol/guanidine thiocyanate solution "TripleXtractor Direct" with a spin column system for convenient, rapid and cost-effective purification of ultrapure RNA without the need of chloroform phase separation and isopropanol precipitation.

Following a one-step homogenization/lysis step with TripleXtractor Direct, in which cells are disrupted and cellular components are dissolved efficiently, while maintaining RNA integrity, the sample is passed through an RNA binding spin column. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. Optional DNase treatment can be included in the protocol. The purified RNA is subsequently eluted by RNase-free water. The entire procedure can be completed in approximately 15 minutes with a typical A260/A280>1.9 and A260/A230 >1.9. The purified total RNA is free of DNA and proteins and can be used for all common downstream applications such as cDNA Library construction, Northern Blotting, RT-PCR, *in vitro* translation, Nuclease Protection Assays, etc.

QC: The quality of the TripleXtractor directRNA kit is tested on a lot-to-lot basis by extracting RNA from a 1 ml *Escherichia coli* culture with an OD of ~1.3 (~10^9 cells). Purified RNA is analyzed by spectrophotometry and by agarose gel electrophoresis.

Caution: This product contains phenol and guanidine isothiocyanate. Always work in fume hood and wear protective clothing and goggles. Toxic in contact with skin and if swallowed. Causes burns. After contact with skin or eyes, wash immediately with plenty of water. If you feel unwell, seek medical advice (show label where possible).

In order to prevent RNase contamination, one should use disposable plasticware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-free and used only for RNA procedures. Glass items can be baked at 150˚C for 4 hours and plastic items can be soaked in 0.5M NaOH for 10 minutes, rinsed thoroughly with RNase-free water and autoclaved. During handling, gloves should be worn at all times.

References:

1. Vogelstein, B., and Gillespie, D. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 615-619

Kit Contents	(100 preps)	(4 preps)
TripleXtractor Direct	80 ml	4 ml
Wash Buffer 1*	35 ml	1.4 ml
Wash Buffer 2*	50 ml	2 ml
RNase-free Water	6 ml	1 ml
RNA mini spin column	100	4
1.5-ml microtube (DNase/RNase free)	200	-
2-ml collection tube	200	8
DNase I solution	0.55 ml	-
DNase I reaction buffer	5 ml	-

Required Components (not included)
Ethanol (96%-100%)
Centrifuge for microtubes
Pipets and tips (RNase-free)
Vortex
β-mercaptoethanol

* Add Ethanol (96%-100%) [not included] to Wash Buffers 1 and 2, as indicated on the bottles, prior to initial use. After Ethanol has been added, mark the bottles to indicate that this step has been completed. Close bottle tightly to avoid ethanol evaporation.

Storage: Transport of DNase I solution and DNase I reaction buffer is carried out either with or without cooling. Upon arrival, both should be stored at -20°C. One should consider to prepare small aliquots, as it is not recommended to repeat thawing and freezing cycles more than 3 times. All other components should be stored at room temperature. Examine solutions for precipitates before use. Any precipitate may be re-dissolved by warming the solution to 37°C followed by cooling to 25°C. Store for up to 2 years.

DNase I treatment of RNA

DNA contamination in the final RNA solution interferes with several downstream applications, such as gene expression analysis. The amount of DNA contamination in the RNA eluate can be significantly reduced by DNase I treatment of the sample. This can be conveniently done "in column" (see step 4 on page 4). We highly recommend to use the reaction buffer included in this kit, as standard DNase buffers often are incompatible with in column DNase I treatment and might compromise RNA yield and integrity. For some very sensitive applications it might be necessary to eliminate even the smallest amounts of residual DNA. In order to effectively remove any trace amounts of DNA, one should consider to treat the eluted RNA with DNase I as described hereunder.

DNA Digestion in Solution (Optional)

Mix as follows in a RNase-free microtube:

- Purified RNA (in RNase-free water): 5-40 µl
- DNase I Reaction Buffer (1x): 5 µl
- DNase I Solution : 0.5 µl for each µg of purified RNA
- RNase-free water: make up to final volume of 50 µl

Incubate at 37°C for 15-30 minutes and stop the reaction by adding 1µl of 20mM EGTA (pH 8.0) and heating at 65°C for 10 minutes.

If desired, DNase I can be removed from the reaction mixture by standard phenol extraction.

SAMPLE PREPARATION

Sample preparation should be done at room temperature. The required volume of TripleXtractor Direct for homogenization and lysis depends on the type of sample.

a. Tissues

Homogenize 10-50 mg tissue samples in 700 µl of TripleXtractor Direct using a tissue homogenizer or rotor-stator. Incubate for 5 minutes at room temperature and transfer the lysate to a 1.5-ml RNase-free microtube. Proceed with RNA Purification.

b. Cells in Suspension

Transfer up to $5x10^6$ cells to a 1.5-ml RNase-free microtube, harvest by centrifugation and discard supernatant. Lyse cells in 700 µl of TripleXtractor Direct by pipetting up and down several times. **DO NOT** wash the cells before addition of TripleXtractor as this increases the possibility of mRNA degradation. Incubate for 5 minutes at room temperature. Proceed with RNA Purification.

c. Adherent Cells

Remove the culture medium and lyse cells directly in the culture dish by adding 100 µl of TripleXtractor Direct per cm^2 of culture area and passing the lysate several time through a pipet. Incubate for 5 minutes at room temperature, and transfer the sample to a 1.5-ml RNase-free microtube. Proceed with RNA Purification.

Note that the amount of TripleXtractor required depends on the area and not on the amount of cells.

d. Body Fluids

Transfer up to 200 µl to a 1.5-ml RNase-free microtube and add 3 volumes of TripleXtractor Direct. Mix well by vortexing. Incubate for 5 minutes at room temperature. Proceed with RNA Purification.

e. Bacteria

Transfer up to $1x10^9$ cells to a 1.5-ml RNase-free microtube. Harvest by centrifugation at 12.000-16.000g for 1-2 minutes and discard the supernatant. Lyse cells in 700 µl of TripleXtractor Direct by pipetting up and down several times. Incubate for 5 minutes at room temperature. Proceed with RNA Purification.

Note: Yield can be increased 2x-3x by pre-treatment of bacterial cells with lysozyme in a Bacterial Lysis Buffer. Lysozyme and Bacterial Lysis Buffer can be acquired from GRiSP separately.

PROTOCOL FOR RNA PURIFICATION

1) Centrifuge the sample at 14,000-16,000g for 1 minute and transfer the clear supernatant to a new 1.5-ml microcentrifuge tube (RNase-free) and add 1 volume of absolute ethanol. Mix by vortexing

2) Place an RNA Binding Column in a 2-ml collection tube and transfer up to 700µl of the sample mixture of step 1 to the column.

3) Centrifuge at 14,000g-16,000g for 60 seconds. Discard the flow-through, and place the spin column back in the collection tube. Transfer the remaining mixture of step 1 to the column and centrifuge 14,000g-16,000g for 60 seconds. Discard the flow-through, and place the spin column back in the collection tube.

4) [**optional (see page 2)**] Add 400 µl of Wash Buffer 2* and centrifuge at 14,000g-16,000g for 30 seconds. Discard the flow-through and place the RNA Binding column back in the collection tube. Mix for each prep 45 µl of DNase I reaction buffer with 5 µl of DNase I solution in a RNase-free tube, and then pipet 50 µl to the center of each spin column. Incubate at room temperature for 10-15 minutes. **Ensure ethanol was added to Wash Buffer 2 prior to use this kit the 1st time.*

5) Add 400 µl of Wash Buffer 1* and centrifuge at 14.000g-16.000g for 30 seconds. Discard the flow-through and place the RNA mini spin column back in the collection tube. Add 600 µl of Wash Buffer 2* and centrifuge at 14.000g-16.000g for 30 seconds. **Ensure ethanol was added to Wash Buffers 1 and 2 prior to use this kit the 1st time.*

6) Discard the flow-through and add 600 µl of Wash Buffer 2* and centrifuge at 14.000g-16.000g for 30 seconds. **Ensure ethanol was added to Wash Buffers 1 and 2 prior to use this kit the 1st time.*

7) Discard the flow-through and place the RNA mini spin column back in the collection tube and centrifuge at 14.000g-16.000g for another 3 minutes to dry the matrix of the column.

8) Transfer spin column to a new 1.5-ml microcentrifuge tube (RNase-free) and pipet 50 µl of RNase-free Water directly to the center of the spin column without touching the membrane. Incubate at room temperature for 1-2 minutes. Total yield can be increased by eluting with larger volume (e.g.,100 µl) whereas concentration can be increased with eluting with smaller volume (e.g., 25 µl) of RNase-free water.

9) Centrifuge for 1 minutes at 14,000g-16,000g to elute purified total RNA. Discard the spin column and use RNA immediately, or store at -20 °C for short term storage or at -70°C for long term storage.

TROUBLESHOOTING

1. **Low Yield**
   - *Clogged Column*
     - **i.** Reduce the amount of sample material.
     - **ii.** Insufficient disruption and/or homogenization.
   - *Incorrect RNA Elution Step*
     - **i.** Ensure that the RNase-free water is completely adsorbed after being added to the center of the spin column.

2. **Low Quality**
   - *Low performance in downstream applications*
     - **i.** Residual ethanol contamination interferes with downstream applications. Following the wash step, dry the spin column with additional centrifugation for 5 minutes or incubation at 60ºC for 5 minutes in order to evaporate ethanol.
     - **ii.** RNA degradation by RNases (which can be detected by gel analysis), may be the result of improper handling of starting material.