LITE total RNA Kit – Blood & Cultured Cells

#GK08Lite (200 preps) | GK08s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample :	up to 300 µl of whole fresh blood, up to 5x10^6 cultured animal cells
Expected Yield :	2-3 µg total RNA (300 µl blood), 20-25 µg (1x10^6 293T cells)
Format :	spin column
Operation Time :	approximately 30 minutes
Elution Volume :	25-100µl

Product: The LITE Total RNA Kit – Blood & Cultured Cells - provides an efficient and fast method for the purification of high-quality total RNA (including mRNA, tRNA and rRNA) from fresh whole blood (human) and cultured animal cells. Eluted purified RNA is suitable for RT-PCR, Northern Blotting, mRNA selection, cDNA synthesis, and primer extension.

The LITE Total RNA Kit – Blood & Cultured Cells - uses detergents and chaotropic salts to lyse cells and to denature proteins/inactivate RNase. Optional DNase treatment can be included in the protocol to remove undesired trace amounts of DNA. The buffer system is optimized to allow selective binding of RNA to the glass fiber matrix of the spin column[1]. Contaminants are completely removed using a Wash Buffer (containing ethanol) in a simple centrifugation step. The purified RNA is subsequently eluted with RNase-free Water. The entire procedure can be completed within 30 minutes with typical RNA yields of 3-30µg.

QC: The quality of the LITE Total RNA Kit – Blood & Cultured Cells - is tested on a lot-to-lot basis by isolating total RNA from 300 µl of whole blood. Quantity and Quality are ascertained by spectroscopy and gel electrophoresis.

Caution: Buffer R1 contains chaotropic salt which is a harmful irritant. During operation, always wear a lab coat, disposable gloves, and protective goggles.

In order to prevent RNase contamination, one should use disposable plastic ware. Automatic pipettes and non-disposable glassware or plasticware should be sterile/RNase-free and used only for RNA procedures. 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	LITE (200 preps)	sample (4 preps)	GRS* (100 preps)
Red Blood Cell Lysis Buffer	400 ml	10 ml	200 ml
Buffer R1	120 ml	2 ml	60 ml
Wash Buffer 1	100 ml	2 ml	50 ml
Wash Buffer 2**	75 ml	1.5 ml	37.5 ml
RNase-free Water	30 ml	1 ml	15 ml
RNA mini spin column	200	4	100
1.5-ml microtube (DNase/RNase free)	-	-	200
2-ml collection tube	400	8	200
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
Water bath or Thermoblock
Ice
β-mercaptoethanol
1.5-ml microtube (DNase/RNase free)

* GRS versions of NAP Kits contain all the necessary components for the standard protocols, including optional steps.
** Add Ethanol (96%-100%) [not included] to Wash Buffer 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.

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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 8 of the blood protocol on page 3 and step 6 of the cultured animal cells protocol 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.

Alternatively, remove DNase I by adding 250 µl of buffer R1 and 300µl of 70% ethanol (prepared with RNase-free water) and mix well by vortexing. Transfer all of the mixture to a new RNA mini spin column and centrifuge at 14,000-16,000g for 1 minute. Discard the flow-through and proceed with step 9 on page 3. Note that following this option the total amount of RNA purifications that can be done with this kit will be reduced.

PROTOCOL FOR TOTAL RNA PURIFICATION FROM FRESH HUMAN BLOOD

1) Transfer up to 300 µl of fresh blood (from a blood collection tube containing EDTA or other anticoagulant) to a 1.5-ml RNase-free microcentrifuge tube, and add 3 volumes of Red Blood Cells Lysis Buffer (e.g., add 750 µl lysis buffer to 250 µl of blood). Mix by inversion.

2) Incubate on ice for 10 minutes (during incubation, vortex twice for a few seconds).
[Red Blood Cell Lysis Buffer Treatment removes non-nucleated red blood cells and reduces hemoglobin contamination]

3) Centrifuge at 3,000g for 5 minutes and remove the supernatant completely.

4) Resuspend the pellet with 100 µl of Red Blood Cell Lysis Buffer add lyse cells by adding 400 µl of Buffer R1 and 4 µl of β-mercaptoethanol. Shake vigorously, and incubate at room temperature for 5 minutes.

5) Add 500 µl of 70% ethanol (prepared with RNase-free ddH_2O) to the sample lysate and mix well by shaking vigorously. In case precipitate appears, break it up by pipetting.

6) Place the RNA mini spin column in a 2-ml collection tube and transfer 500 µl of the sample mixture (including any precipitate if present) to the column.

7) Centrifuge at 14,000g-16,000g for 1 minute. Discard the flow-through and transfer the remaining sample to the same spin column. Centrifuge at 14,000g-16,000g for 1 minute. Discard the collection tube containing the flow-through and place the RNA mini spin column in a new collection tube.

8) ***[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 mini spin 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.*

9) 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 Buffer 2 prior to use this kit the 1st time.*

10) 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 Buffer 2 prior to use this kit the 1st time.*

11) 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.

12) Transfer the 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.

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

PROTOCOL FOR RNA PURIFICATION FROM CULTURED ANIMAL CELLS

(In case of using adherent cells, trypsinize cells before harvesting)

1) Transfer up to $5x10^6$ cultured animal cells to a 1.5-ml microcentrifuge tube (RNase-free) and centrifuge for 5 minutes at 300g to harvest the cells. Discard the supernatant.

2) Resuspend the pellet with 100 µl of Red Blood Cell Lysis Buffer and lyse cells by adding 400 µl of Buffer R1 and 4 µl of β-mercaptoethanol. Mix well by shaking vigorously. Incubate at room temperature for 5 minutes.

3) Add 500 µl of 70% ethanol (prepared with RNase-free ddH_2O) to the sample lysate and mix well by shaking vigorously. In case precipitate appears, break it up by pipetting.

4) Place the RNA mini spin column in a 2-ml collection tube and transfer 500 µl of the sample mixture (including any precipitate if present) to the column.

5) Centrifuge at 14,000g-16,000g for 1 minute. Discard the flow-through and transfer the remaining sample to the same spin column. Centrifuge at 14,000g-16,000g for 1 minute. Discard the collection tube containing the flow-through and place the RNA mini spin column in a new collection tube.

6) ***[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 mini spin 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.*

7) 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 Buffer 2 prior to use this kit the 1st time.*

8) 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 Buffer 2 prior to use this kit the 1st time.*

9) 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.

10) Transfer the 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 improved by eluting with larger volume (e.g., 100µl) whereas concentration can be increased by eluting with smaller volume (e.g., 25µl) of RNase-free water.

11) Centrifuge for 1 minute 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.
   - *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.