LITE total RNA Kit – Tissue

#GK09Lite (200 preps) | GK09s (trial size, 4 preps)
(FOR RESEARCH ONLY)

Sample : up to 25 mg of tissue, up to 25 mg of paraffin-embedded tissue
Expected Yield : 5-30 µg total RNA
Format : spin column
Operation Time : approximately 30 minutes
Elution Volume : 25-100µl

Product: The LITE Total RNA Kit - Tissue - provides an efficient and fast method for the purification and/or concentration of high-quality total RNA (including mRNA, tRNA and rRNA) from a variety of animal and paraffin-embedded tissues. Optional DNase treatment can be included in the protocol to remove undesired DNA residue. Eluted purified RNA is suitable for RT-PCR, Northern Blotting, mRNA selection, cDNA synthesis, and primer extension.

The LITE Total RNA Kit - Tissue - uses detergents and chaotropic salts to lyse cells and to denature proteins/inactivate RNase. (If desired, an optional DNase treatment step can be followed in order to remove unwanted residual 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 5-30µg.

QC: The quality of the LITE Total RNA Kit - Tissue - is tested on a lot-to-lot basis by isolating total RNA from 25 mg animal tissue sample. 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)	Required Components (not included)
Buffer R1	120 ml	2 ml	60 ml	Ethanol (96%-100%)
Wash Buffer 1	100 ml	2 ml	50 ml	Centrifuge for microtubes
Wash Buffer 2**	75 ml	1.5 ml	37.5 ml	Pipets and tips (RNase-free)
RNase-free Water	30 ml	1 ml	15 ml	Vortex
RNA mini spin column	200	4	100	Water bath or Thermoblock
Filter column	200	4	100	Ice
1.5-ml microtube (DNase/RNase free)	-	-	200	β-mercaptoethanol
2-ml collection tube	400	8	200	Xylene
Micropestle	200	4	100	20-G needle syringe
Proteinase K***	-	1mg	2x 11mg	1.5-ml microtube (DNase/RNase free)
DNase I solution		-	0.55 ml	
DNase I reaction buffer		-	5 ml	

* 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.

*** Add Water (RNase-free) to Proteinase K, as indicated on the tube, prior to initial use. After Water has been added, mark the tube to indicate that this step has been completed.

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. Proteinase K is a stable enzyme and transport is carried out either with or without cooling. Upon arrival, proteinase K (powder) should be stored at 4°C. Once water has been added, it is recommended to store the Proteinase K solution at -20°C. 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 on page 3). 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 TISSUE

1) Cut up to 25mg of fresh or frozen animal tissue and transfer up to a 1.5-ml RNase-free microcentrifuge tube. [Notes: **a)** If using frozen animal tissue, the sample must have been flash frozen in liquid nitrogen and immediately stored at -70ºC until use to prevent RNA degradation. **b)** In case of some tissues, like spleen that contain a very high number of cells, the starting material should be reduced to 10mg].

2) Add 400µl of Buffer R1 and 4µl of β-mercaptoethanol. Use the micropestle (provided) to grind the tissue. Shear the tissue passing the lysate 10 times through a 20-G needle syringe (not provided)

3) Incubate at room temperature for 5 minutes.

4) Place a filter column in a 2-ml collection tube and transfer the sample mixture to the filter column. Centrifuge at 1,000g for 1 minute. Discard the filter column.

5) Add 400µl of 70% ethanol (prepared with RNase-free ddH_2O) to the filtrate 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 500µl of the sample mixture (including any precipitate) to the column

7) Centrifuge at 14,000g-16,000g for 2 minutes. If the lysate did not pass completely through the column, increase centrifuge time until the mixture passes completely. Discard the flow-through and transfer the remaining sample to the same spin column. Centrifuge at 14,000g-16,000g for 2 minutes (or longer if needed). Discard the collection tube 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 PARAFFIN-EMBEDDED TISSUE

1) Slice small sections, up to 25 mg, from blocks of paraffin-embedded tissue and transfer to a 1.5-ml microcentrifuge tube (RNase-free).

2) Add 1ml of Xylene. Vortex vigorously and incubate at room temperature for 10 minutes. During incubation, vortex regularly.

3) Centrifuge at 14,000g-16,000g for 3 minutes. Discard the supernatant.

4) Add 1 ml of absolute ethanol to wash the pellet. Mix by inverting. Repeat steps 3 and 4.

5) Centrifuge at 14.000g-16.000g for 3 minutes. Remove the supernatant and incubate the tube with open lid at 37°C to evaporate any residual ethanol. Proceed with step 2 of the Tissue protocol.

PROTOCOL FOR RNA PURIFICATION FROM ESPECIALLY DIFFICULT TISSUE

1) Slice small sections, up to 25 mg of tissue and transfer to a 1.5-ml microcentrifuge tube (RNase-free).

2) Add 80 µl of RNAse-free water and 20 µl of Proteinase K (10mg/ml) and grind the tissue using the provided micropestle.

3) Incubate at 60°C for 20 minutes. Add 400 µl of Buffer R1 and 4 µl of β-mercaptoethanol, and continue with step 3 of the Tissue protocol.

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 60°C 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.