96-Well Total RNA Kit

For research use only

Sample : cultured animal cells (up to 5 x 10^6)

Yield : up to 30 µg/well

Format : 96-well plates

Operation : centrifuge/vacuum manifold

Applications : RT-PCR, Real-time PCR, Northern Blotting, mRNA selection, Microarrays, cDNA Synthesis

www.geneaid.com

Introduction

The 96-Well Total RNA Kit was designed specifically for high-throughput purification of total RNA from cultured animal cells. Using either vacuum or centrifuge, RNA preparations of 96 samples can be completed in 1 hour. Chaotropic salt is used to lyse cells and inactivate RNase allowing RNA to be easily bound by the glass fibre matrix (1) of each well of the plate. Once any contaminants have been removed, using the Wash Buffer (containing ethanol) the purified RNA is eluted by RNase-free water. The purified RNA is ready for use in various downstream applications.

Quality Control

The quality of the 96-Well Total RNA Kit is tested on a lot-to-lot basis by isolating total RNA from cultured cell samples. The purified RNA is quantified with a spectrophotometer and checked by electrophoresis.

Kit Contents

Name	RBP02	RBP04	RBP10
RB Buffer	60 ml	100 ml	240 ml
W1 Buffer	130 ml	130 ml	130 ml x 3
Wash Buffer[1] (Add Ethanol)	50 ml (200 ml)	50 ml x 2 (200 ml x 2)	50 ml x 6 (200 ml x 6)
RNase-free water	30 ml	60 ml	120 ml
RNA Binding Plate	2 pcs	4 pcs	10 pcs
0.35 ml Collection Plate	2 pcs	4 pcs	10 pcs
Adhesive film	4 pcs	8 pcs	20 pcs

Order Information

Product Name	Package size	Cat. No.
Total RNA Mini Kit (Blood/Cultured Cell)	50/100/300 preps	RB050/100/300
Total RNA Maxi Kit (Blood/Cultured Cell)	10/25 preps	RBM10/25
Total RNA Mini Kit (Tissue)	50/100/300 preps	RT050/100/300
Total RNA Maxi Kit (Tissue)	10/25 preps	RTM10/25
Total RNA Mini Kit (Plant)	50/100/300 preps	RP050/100/300
Total RNA Maxi Kit (Plant)	10/25 preps	RPM10/25
miRNA Isolation Kit	100 preps	PU009100
96-Well Total RNA Kit	2/4/10 x 96 Wells	RBP02/04/10

[1]Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use

Caution

Buffers contain irritant agents. During operation, always wear a lab coat, disposable gloves, and protective goggles.

References

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

96-Well Total RNA Kit Vacuum Protocol

The provided protocol is for up to 5 x 10^6 cell use. If more than 5 x 10^6 cells are to be used, transfer them to a 2 ml collection plate and proportionally increase the volume of the lysis buffer (RB Buffer) and 70% ethanol before the Binding Step (the buffer volume in the Wash and Elution Steps do not require any increase).

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: multi-well plate vacuum manifold, 2 ml collection plate (RNase-free), absolute ethanol, ß-mercaptoethanol

Step1 Harvesting	**Monolayer cell culture** • Incubate cells in multi-well culture plates. • Remove the culture medium by pipetting. **Suspension cell culture** • Transfer cells (up to 5 x 10^6) into each well of a 2 ml collection plate. • Spin the cells for 5 minutes at 300 x *g*, and remove the supernatant by pipetting.
Step 2 Cell Lysis	• Add **200 µl of RB Buffer** and 2 µl of ß-mercaptoethanol to each well of the 2 ml collection plate. • Lyse the cells by shaking or pipetting. • Incubate at room temperature for 5 minutes or until the lysate clears.
Step 3 RNA Binding	• Add 200 µl of 70% ethanol to each sample lysate in the 2 ml collection plate. • Seal the plate with an **Adhesive Film** and mix immediately by vortex. • Lift up the top portion of the vacuum manifold and place a new 2 ml collection plate on the base and reassemble the vacuum manifold. • Place a **RNA Binding Plate** onto the top gasket of the vacuum manifold and fit both together tightly. • Remove the **Adhesive Film** from the 2 ml collection plate and transfer all of the lysate mixture to each well of the **RNA Binding Plate.** • Turn on the vacuum pump at 800 mbar for a few seconds or until the wells empty.
Step 4 Wash	• Turn off the vacuum pump and add **300 µl of W1 Buffer** to each well of the **RNA Binding Plate**. • Turn on the vacuum pump at 800 mbar for a few seconds or until the wells empty. • Turn off the vacuum pump and add **600 µl of Wash Buffer** (ethanol added) to the **RNA Binding Plate.** • Turn on the vacuum pump at 800 mbar for 1 minute. • Turn off the vacuum pump and discard the flow-through from the 2 ml collection plate. • Return the **RNA Binding Plate** and collection plate to the vacuum manifold. • Add **600 µl of Wash Buffer** (ethanol added) to each well of the **RNA Binding Plate**. • Turn on the vacuum pump at 800 mbar for 1 minute. • Turn off the vacuum pump and lift the **RNA Binding Plate** from the manifold and press it on an absorbent material to blot out the excess liquid from the bottom of the plate. • Return the **RNA Binding Plate** and 2 ml collection plate to the vacuum manifold. • Turn on the vacuum pump at 800 mbar for 10 minutes to dry the plate membrane.
Step 5 RNA Elution	• Turn off the vacuum pump and lift up the top portion of the vacuum manifold carrying the **RNA Binding Plate** and remove the 2 ml collection plate containing the flow-through waste. • Place a **0.35 ml Collection Plate** on the base and reassemble the vacuum manifold with the **RNA Binding Plate**. • Add **70 µl of RNase-free water** to the center of each well of the **RNA Binding Plate**. • Let stand for 3 minutes or until the water is absorbed by the plate matrix. • Turn on the vacuum pump at 800 mbar for a few seconds to elute the viral DNA/RNA. • Turn off the vacuum pump and remove the **0.35 ml Collection Plate** containing the eluted product**.** • Seal the plate with a new **Adhesive Film** and store the purified RNA at -20ºC.

96-Well Total RNA Kit Centrifuge Protocol

The provided protocol is for up to 5 x 10^6 cell use. If more than 5 x 10^6 cells are to be used, transfer them to a 2 ml collection plate and proportionally increase the volume of the lysis buffer (RB Buffer) and 70% ethanol before the Binding Step (the buffer volume in the Wash and Elution Steps do not require any increase).

- Add absolute ethanol (see the bottle label for volume) to the Wash Buffer prior to initial use
- Additional requirements: 2 ml collection plate (RNase-free), absolute ethanol, ß-mercaptoethanol

Step1 Harvesting	**Monolayer cell culture** • Incubate cells in multi-well culture plates. • Remove the culture medium by pipetting. **Suspension cell culture** • Transfer cells (up to 5 x 10^6) into each well of a 2 ml collection plate. • Spin the cells for 5 minutes at 300 x *g*, and remove the supernatant by pipetting.
Step 2 Cell Lysis	• Add **200 µl of RB Buffer** and 2 µl of ß-mercaptoethanol to each well of the 2 ml collection plate. • Lyse the cells by shaking or pipetting. • Incubate at room temperature for 5 minutes or until the lysate clears.
Step 3 RNA Binding	• Add 200 µl of 70% ethanol to each sample lysate in the 2 ml collection plate. • Seal the plate with **Adhesive Film** and vortex immediately. • Place a **RNA Binding Plate** on a 2 ml Collection Plate. • Remove the film and transfer all of the lysate mixture from Step 2 to each well of the **RNA Binding Plate**. • Centrifuge for 5 minutes at 1,000 x g. • Discard the flow-through and place the **RNA Binding Plate** back on the 2 ml Collection Plate.
Step 4 Wash	• Add **300 µl of W1 Buffer** to the **RNA Binding Plate**. • Centrifuge for 5 minutes at 1,000 x g. • Add **600 µl of Wash Buffer** (ethanol added) to the **RNA Binding Plate**. • Centrifuge for 3 minutes at 1,000 x g. • Discard the flow-through and place the **RNA Binding Plate** back on the 2 ml Collection Plate. • Add **600 µl of Wash Buffer** (ethanol added) to the **RNA Binding Plate**. • Centrifuge for 3 minutes at 1,000 x g. • Discard the flow-through and place the **RNA Binding Plate** back on the 2 ml Collection Plate. • Centrifuge for 10 minutes at 1,000 x g to remove ethanol residue.
Step 5 RNA Elution	• Transfer the **RNA Binding Plate** to a clean **0.35 ml Collection Plate**. • Add **70 µl of RNase-free water** to the center of each well of the **RNA Binding Plate**. • Let Stand for 3 minutes or until the water is absorbed by the matrix. • Centrifuge for 5 minutes at 1,000 x g to elute the purified RNA. • Seal the plate with a new **Adhesive Film** and store the purified RNA at -20℃.