HiYield™ 96-Well Total RNA Extraction Kit

Cat. No.:	YRB96B-2	YRB96B-4	YRB96B-10
Product Name:	HiYield™ 96-Well Total RNA Extraction Kit		
Reactions:	2	4	10
Format:	96-Well Plates		
Binding Capacity:	Up to 30 ug/well		
Operation:	Centrifuge or Vacuum		
Operation Time:	Within 60 Minutes		

Introduction

HiYield™ 96-Well Total RNA Extraction Kit is especially designed for high-throughput purification of total RNA from up to 5 x 10^6 Cultured Cells. With centrifuge or vacuum manifold, RNA preparation of 96 samples can be completed in one hour. The purified RNA is ready for use in various downstream applications.

Features

High-throught purification of total RNA.
Ready-to-use RNA within 60 minutes.
Reproducible recoveries.

Applications

Purified RNA is ready for direct use in RT-PCR, Quantitative RT-PCR, cDNA Synthesis, Northern Blot Analysis, Primer Extension, mRNA Selection, Microarrays.

Quality Control

The quality of the HiYield™ 96-Well Total RNA Extraction 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.

Contents

ITEM	YRB96B-2	YRB96B-4	YRB96B-10
RNA Binding Plate	2 plates	4 plates	10 plates
Adhesive Film	4 pcs	8 pcs	20 pcs
350 µl Collection Plate	2 plates	4 plates	10 plates
RB Buffer	60 ml	100 ml	240 ml
W1 Buffer	130 ml	130 ml	390 ml
Wash Buffer(concentrated)	50 ml	100 ml	300 ml
RNase-Free Water	30 ml	60 ml	120 ml

*Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

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.

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).

Additional Requirements:
Multi-well plate vacuum manifold (ex: HiYield™ 96-Well Vacuum Manifold), 2 ml collection plate (RNase-free), absolute ethanol, ß-mercaptoethanol.

Things Before Starting:
Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

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 **350 µl 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 **350 µl Collection Plate** containing the eluted product**.** ★Seal the plate with a new **Adhesive Film** and store the purified RNA at -20ºC.

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).

Additional Requirements:
2 ml collection plate (RNase-free), absolute ethanol, ß-mercaptoethanol.

Things Before Starting:
Add absolute ethanol to the Wash Buffer prior to initial use (see the bottle label for volume).

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 **350 µl 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℃.