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HiYield™ Total RNA Mini Kit (Bacteria)

Cat. No.:	YRC50	YRC100	YRC300
Product Name:	HiYield™ Total RNA Mini Kit (Bacteria)		
Reactions:	50	100	300
Sample:	Up to 1 x 10^9 of Gram-negative and Gram-positive bacteria		
Yield:	Up to 60µg (1 x 10^9 Escherichia coli: 40-45 µg or 1 x 10^9 Bacillus subtilis: 50-55 µg)		
Elution Volume:	50-100 µl		
Format:	Spin Column		
Operation:	Centrifuge		
Operation Time:	Within 20 Minutes		

Description

HiYield™ Total RNA Mini Kit (Bacteria) is ideal for purification of total RNA from Gram positive and Gram negative bacterial cells in a simple spin column format. Lysozyme and Bacteria Lysis Buffer are included in the kit to efficiently lyse bacterial cell walls consisting of the peptidoglycan layer. Up to 60µg of total RNA can be purified within 20 minutes without phenol/chloroform extraction or alcohol precipitation. Typical yield is around 40-45 µg from 1 x 10^9 of Escherichia coli. The convenient purification system removes contaminants well. Purified RNA is ready for use in RT-PCR, Northern Blotting, Primer Extension, mRNA Selection and cDNA Synthesis.

Features

1. No need for liquid nitrogen, dry ice, or phenol.
2. Reliable isolation of ready-to-use RNA within 20 minutes.
3. Complete removal of all contaminants for sensitive downstream applications.

Applications

Purified RNA is ready for use in RT-PCR, Northern Blotting, Primer Extension, mRNA Selection and cDNA Synthesis.

Quality Control

The quality of HiYield™ Total RNA Mini Kit (Bacteria) is tested on a lot-to-lot basis by isolation of total RNA from 1 x 10^9 of Escherichia coli culture (OD600=1.3, 1 ml) harvested by centrifugation at 16,000 x g for 1 minute. A 10 µl aliquot of purified RNA from a 50 µl eluate is analyzed by electrophoresis on a 0.8% agarose gel and the purified RNA with A260/A280 ratio 1.8 - 2.0 was quantified with a spectrophotometer.

Contents

Item	YRC50	YRC100	YRC300
Bacteria Lysis Buffer	15 ml	30 ml	75 ml
Lysozyme*	220 mg	420 mg	1220 mg
RB Buffer	30 ml	60 ml	130 ml
W1 Buffer	30 ml	50 ml	130 ml
Wash Buffer (concentrated)**	12.5 ml	25 ml	75 ml
RNase-free Water	6 ml	6 ml	30 ml
RB Column	50 pcs	100 pcs	300 pcs
2 ml Collection Tube	100 pcs	200 pcs	600 pcs

* Lysozyme should be stored at -20ºC for extended periods. Add Lysozyme to Bacteria Lysis Buffer immediately prior to use. Once Lysozyme is mixed with Bacteria Lysis Buffer, the solution can be stored for 2 weeks at 4ºC.

**Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Storage

HiYield™ Total RNA Mini Kit (Bacteria) shall be shipped and stored dry at room temperature (15-25℃). With proper storage, HiYield™ Total RNA Mini Kit (Bacteria) can be stored for up to 9 months without showing any deduction in performance and quality. Lysozyme should be stored at -20ºC for extended periods

Important Notes

Please read the entire notes prior to starting any of the protocol procedures.

Caution:

HiYield™ Total RNA Mini Kit (Bacteria) contains irritants. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.

Reagents to Be Supplied by User:

Absolute ethanol, ddH_2O (RNase-free and DNase-free) to prepare 70% ethanol, microcentrifuge tubes (RNase-free), 15 ml centrifuge tube (RNase-free), pipette tips (RNase-free) and ß-mercaptoethanol.

Things to Do before Starting:

1. Add Lysozyme to Bacteria Lysis Buffer immediately prior to use. Once Lysozyme is mixed with Bacteria Lysis Buffer, the solution can be stored for 2 weeks at 4ºC.
2. Add absolute ethanol (see the bottle label for volume) to Wash Buffer then mix by shaking for a few seconds. Check the box on the bottle showing absolute ethanol is added. Be sure to close the bottle tightly after each use to avoid ethanol evaporation.

Steps to Prevent RNase Contamination:

1. During operation, always wear a lab coat, disposable gloves, protective goggles and (anti-fog) procedure mask.
2. Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures.
3. Non-disposable glassware or plasticware should also be sterile (RNase-free).

DNA Removal Options:

For DNA-free RNA, perform optional steps listed in the protocol. Either follow the steps after RNA Binding, or steps after RNA Elution.

Protocol

Please read the entire important notes prior to starting.

Step 1 Sample Preparation	1. Transfer bacterial cells (up to 1 x 10^9) to a 1.5 ml microcentrifuge tube (RNase-free). 2. Centrifuge for 1 minute at 14,000-16,000 x g then remove the supernatant completely. 3. Transfer required volume of Bacteria Lysis Buffer (200 µl/sample) to a 15 ml centrifuge tube (RNase-free). Add Lysozyme to Bacteria Lysis Buffer immediately prior to use. 4. Add Lysozyme (20 mg/ml) to Bacteria Lysis Buffer (in the 15 ml centrifuge tube) and vortex to completely dissolve the Lysozyme. 5. Transfer 200 µl of Bacteria Lysis Buffer (make sure Lysozyme was added) to the sample in the 1.5 ml microcentrifuge tube then re-suspend the pellet by pipetting. 6. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.
Step 2 Cell Lysis	1. Add 300 µl of RB Buffer and 3 µl ß-mercaptoethanol to the sample lysate from Step 1 then vortex to mix. Incubate the mixture at room temperature for 5 minutes and then centrifuge at 14,000-16,000 xg for 2 minutes. 2. Transfer the supernatant to a new 1.5 ml microcentrifuge tube (RNase-free).
Step 3 RNA Binding	1. Add 500 µl of 70% ethanol to the lysate and pipette immediately. 2. Place a RB Column in a 2 ml Collection Tube and transfer 500 µl of the mixture to the RB Column. Centrifuge at 14,000-16,000 x g for 1 minute then discard the flow-through. 3.Transfer the remaining mixture to the same RB Column and centrifuge at 14,000-16,000 xg for 1 minute. Discard the flow-through and place the RB Column in a new 2 ml Collection Tube. DNA Removal Option 1: Add 100 µl of DNase I (2 KU/ml) mixed in a reaction buffer (50 mM Tris-HCl pH 7.5, 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC) to the center of the RB Column matrix. Let stand for 10 minutes at room temperature and then proceed to Step 4 Wash.

Step 4 Wash	1. Add 400 µl of W1 Buffer to the RB Column then centrifuge at 16,000 x g for 1 minute. 2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube. 3. Add 600 µl of Wash Buffer (ethanol added) into the RB Column. 4. Centrifuge at 14,000-16,000 x g for 1 minute. Discard the flow-through then place the RB Column back in the 2 ml Collection Tube. 5. Add 600 µl of Wash Buffer (ethanol added) into the RB Column. Centrifuge at 14,000-16,000 x g for 1 minute. Discard the flow-through then place the RB Column back in the 2 ml Collection Tube. 6. Centrifuge at 14,000-16,000 x g for 3 minutes to dry the column matrix.
Step 5 RNA Elution	1. Place the dried RB Column in a clean 1.5 ml microcentrifuge tube (RNase-free). 2. Add 50 µl of RNase-free Water into the CENTER of the column matrix. Let stand for at least 3 minutes to ensure the RNase-free Water is absorbed by the matrix. 3. Centrifuge at 14,000-16,000 x g for 1 minute to elute the purified RNA. DNA Removal Option 2: Add 2 µl of DNase I (2 KU/ml) mixed in a reaction buffer (50 mM Tris-HCl pH 7.5, 10 mM $MnCl_2$, 50 µg/ml BSA at 25ºC) to the final elution sample. Let stand for 10 minutes at room temperature.

Troubleshooting

Problem	Possible Reasons/Solution
Clogged Column	1. Reduce the amount of starting material or separate it into multiple tubes. 2. Centrifugation temperature must be between 20ºC to 25ºC. 3. Bacteria cells were not completely homogenized. 4. Make sure Lysozyme was added to Bacteria Lysis Buffer immediately prior to use and make sure the mixture of Lysozyme and Bacteria Lysis Buffer was stored at 4ºC for less than 2 weeks.
Residual Ethanol Contamination	Following the wash step, dry the RB Column with additional centrifugation at 14,000-16,000 x g for 5 minutes.
RNA Degradation	1. The harvested sample should be stabilized immediately prior to use. 2. Disposable plasticware and automatic pipettes should be sterile (RNase-free) and used only for RNA procedures. 3. Non-disposable glassware or plasticware should also be sterile (RNase-free).