Presto™ Mini RNA Bacteria Kit Quick Protocol

For research use only

Catalogue Number

RBB004, RBB050, RBB100, RBB300, RBBD004, RBBD050, RBBD100, RBBD300

Instruction Manual Download

When using this product for the first time, or if you are unfamiliar with the procedure, please scan the QR code and download the complete instruction manual.

Instruction Manual Download

IMPORTANT BEFORE USE!

1. 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. Be sure and close the bottle tightly after each use to avoid ethanol evaporation.

2. Lysozyme and DNase I are shipped at room temperature and should be stored at -20ºC for extended periods.

1. Sample Preparation

Transfer **bacterial cells (up to 1 x 10^9)** to a 1.5 ml microcentrifuge tube (RNase-free). Centrifuge for 1 minute at 14-16,000 x g then remove the supernatant completely. Transfer required volume of Bacteria Lysis Buffer (200 µl/sample) to a 15 ml centrifuge tube (RNase-free). Add **Lysozyme (2 mg/200 µl) to Bacteria Lysis Buffer** (in the 15 ml centrifuge tube) and vortex to completely dissolve the Lysozyme. 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. Incubate at room temperature for 10 minutes. During incubation, invert the tube every 2-3 minutes.

2. Lysis

Add **300 µl of RB Buffer and 3 µl ß-mercaptoethanol** then vortex. Incubate at room temperature for 5 minutes then centrifuge at 14-16,000 x g for 2 minutes. Transfer the supernatant to a new 1.5 ml microcentrifuge tube (RNase-free).

3. RNA Binding

Add **500 µl of 70% ethanol** and pipette immediately. Place a **RB Column** in a 2 ml Collection Tube. Transfer **500 µl of the mixture to the RB Column**. Centrifuge at 14-16,000 x g for 1 minute then discard the flow-through. Place the **RB Column** back in the 2 ml Collection Tube. Transfer the **remaining mixture to the same RB Column** and centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through and place the **RB Column** in a new 2 ml Collection Tube.

Optional Step 1: In Column DNase I Digestion

The amount of DNA contamination is significantly reduced following In Column DNase I Digestion. However, traces of residual DNA may be detected in very sensitive applications. In this situation, please perform Optional Step 2: DNA Digestion In Solution instead to efficiently remove trace amounts of DNA. Standard DNase buffers are incompatible with In Column DNase I Digestion and may affect RNA integrity and reduce yield.

1. Add 400 µl of Wash Buffer (make sure ethanol was added) to the RB Column then centrifuge at 14-16,000 x g for 30 seconds.

2. Discard the flow-through and place the RB Column back in the 2 ml Collection Tube.

3. Prepare DNase I solution in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

DNase I	5 µl (2 U/µl)
DNase I Reaction Buffer	45 µl
Total Volume	50 µl

4. Gently pipette DNase I solution to mix (DO NOT vortex) then add DNase I solution (50 µl) into the CENTER of the RB column matrix.

5. Incubate the column for 15 minutes at room temperature (20-30ºC) then proceed with RNA Wash.

4. Wash

Add **400 µl of W1 Buffer to the RB Column** then centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through and place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer** (make sure ethanol was added) into the **RB Column**. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Add **600 µl of Wash Buffer** (make sure ethanol was added) into the **RB Column**. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow-through then place the **RB Column** back in the 2 ml Collection Tube. Centrifuge at 14-16,000 x g for 3 minutes to dry the column matrix.

5. RNA Elution

Place the dried **RB Column** in a clean 1.5 ml microcentrifuge tube (RNase-free). 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. Centrifuge at 14-16,000 x g for 1 minute to elute the purified RNA.

Optional Step 2: DNA Digestion In Solution

1. Prepare DNase I reaction in a 1.5 ml microcentrifuge tube (RNase-free) as follows:

RNA in RNase-free Water	1-40 µl
DNase I	0.5 µl/µg RNA
DNase I Reaction Buffer	5 µl
RNase-free Water	Add to final volume = 50 µl
Total Volume	50 µl

2. Gently pipette the DNase I reaction solution to mix (DO NOT vortex) then incubate the microcentrifuge tube at 37ºC for 15-30 minutes.

3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0) then incubate the microcentrifuge tube at 65°C for 10 minutes.

4. Repurify the RNA sample by adding 250 µl of RB Buffer to the 50 µl DNase I reaction mixture then mix well by vortex. Add 300 µl of 70% ethanol then mix well by vortex. Transfer all of sample mixture to a new RB Column. Centrifuge at 14-16,000 x g for 1 minute. Discard the flow through. Proceed with the RNA Wash step.

Kit Components

Component	RBB004 RBBD004	RBB050 RBBD050	RBB100 RBBD100	RBB300 RBBD300
Bacteria Lysis Buffer	1.5 ml	15 ml	30 ml	75 ml
Lysozyme[1]	8 mg	110 mg	250 mg	610 mg
RB Buffer	2 ml	30 ml	60 ml	130 ml
DNase I[2] (2U/µl) (RBBD004/050/100/300 Only)	20 µl	275 µl	550 µl	550 µl x 3
DNase I Reaction Buffer (RBBD004/050/100/300 Only)	200 µl	2.5 ml	5 ml	15 ml
W1 Buffer	2 ml	30 ml	50 ml	130 ml
Wash Buffer[3] (Add Ethanol)	1.5 ml (6 ml)	25 ml (100 ml)	25 ml + 12.5 ml (100 ml) (50 ml)	50 ml x 2 (200 ml x 2)
RNase-free Water	1 ml	6 ml	15 ml	30 ml
RB Columns	4	50	100	300
2 ml Collection Tubes	8	100	200	600

[1,2]Lysozyme and DNase I are shipped at room temperature and stored at -20ºC for extended periods after receiving the kit.

[3]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. Be sure and close the bottle tightly after each use to avoid ethanol evaporation. The additional **Wash Buffer x 12.5 ml** is **only** included in **RBBD100**.

Storage

Dry at room temperature (15-25ºC). Lysozyme and DNase I are shipped at room temperature and should be stored at -20ºC for extended periods after receiving the kit.