Presto™ Mini RNA Yeast Kit Quick Protocol

For research use only

Catalogue Number

RBY004, RBY050, RBY100, RBY300, RBYD004, RBYD050, RBYD100, RBYD300

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

Additional Requirements

lyticase or zymolase, absolute ethanol, ddH_2O (RNase-free and DNase-free) to prepare 70% ethanol, microcentrifuge tubes (RNase-free), pipette tips (RNase-free), ß-mercaptoethanol, EGTA (for DNA Digestion In Solution)

1. Cell Harvesting

Transfer **fungus cells (up to 5 x 10^7)** to a 1.5 ml microcentrifuge tube (RNase-free). Harvest fungus cells by centrifugation for 10 minutes at 5,000 x g. Discard the supernatant then re-suspend the pellet in **600 µl of Sorbitol Buffer**. Add **200 U of lyticase or zymolase**. Incubate at 30ºC for 30 minutes. Centrifuge the mixture for 10 minutes at 2,000 x g to harvest the spheroplast then remove the supernatant.

2. Lysis

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 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** to the lysate 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 the RNA Wash step.

4. RNA 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 at 37ºC for 15-30 minutes.
3. Stop the reaction by adding 1 µl of 20 mM EGTA (pH=8.0). 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	RBY004 RBYD004	RBY050 RBYD050	RBY100 RBYD100	RBY300 RBYD300
Sorbitol Buffer	4.5 ml	45 ml	45 ml x 2	225 ml
RB Buffer	2 ml	30 ml	60 ml	130 ml
DNase I[1] (2U/µl) (RBYD004/050/100/300 Only)	20 µl	275 µl	550 µl	550 µl x 3
DNase I Reaction Buffer (RBYD004/050/100/300 Only)	200 µl	2.5 ml	5 ml	15 ml
W1 Buffer	2 ml	30 ml	50ml	130 ml
Wash Buffer[2] (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]DNase I is shipped at room temperature and stored at -20ºC for extended periods after receiving the kit.

[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. 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 **RBYD100**.

Storage

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