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EZ-10 Spin Column Total RNA Mini-preps Super Kit

BS583 & BS584 & BS784

Version 12.1
ISO9001 Certified

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EZ-10 Spin Column Total RNA Minipreps Super Kit

Product Information for BS583 & BS584 &BS784

Kit Contents

Components	BS583 20 Preps	BS584 100 Preps	BS784 250 Preps
RLT Solution[a]	14 ml	70 ml	175 ml
RW Solution	12 ml	60 ml	150 ml
RPE Solution[b]	5 ml	22 ml	2X30 ml
RNase-free Water	1 ml	5 ml	12.5ml
EZ-10 Spin Column & 2.0-ml Collection Tube	20 1	100 1	250 1

(a) RLT Solution should be kept at 2-8^{o}C. It may form a precipitate upon storage. If necessary, dissolve the precipitate by warming the solution at 37^{0}C.

(b) Before use, add 20 ml of 100% of ethanol to 5 ml RPE Solution for BS583, 88 ml of 100% ethanol to 22 ml RPE Solution for BS584, or 120 ml of 100% ethanol to 30 ml RPE Solution for BS784. For other volumes of RPE Solution, simply add enough ethanol to make a 4:1 ratio (volume of added ethanol: volume of RPE Solution = 4:1).

Storage:

The kit is stable for 18 months at room temperature. For longer storage, keep all contents cold.

Note: To remove DNA from RNA, digest RNA from step 7 with DNASE I, RNASE FREE (Catalogue # DD0649). Follow procedures for RNA cleanup below to obtain DNA-FREE RNA.

Procedure for RNA Cleanup

This kit can also be used to clean up RNA, which is isolated by different methods or after enzymatic reactions.

1. Adjust sample to a volume of 100 µl with RNase-free H_2O, add 350 µl of RLT Solution, and mix well. Add 260 µl of 100% ethanol, mix gently. A precipitate may form by adding ethanol, but this will not affect the procedure.
2. Place an EZ-10 Spin Column in 2-ml Collection Tube and transfer the mixture solution (Step 1) to the column and spin at 4,000 rpm (3,500 x g) for 1 minute, discard flow-through.
3. Add 500 µl of RW Solution to the column and centrifuge at 8,000 rpm (6,000 x g) for 1 minute. Discard the solution in collection tube.
4. Add 500 µl of RPE Solution to the column and spin at 8,000 rpm (6,000 x g) for 1 minute, discard the flow-through and spin once more to completely remove the residue of RPE Solution.
5. Add 30-50 µl of RNase-free H_2O onto the center part of the membrane of the column and centrifuge at 10,000 rpm (6,000 x g) for 1 minute. Keep RNA sample at -70℃.

Principle

This kit is designed for fast isolation of total RNA from bacteria, yeast, fungi, plant and animal tissues. The kit contains a membrane embedded spin column for binding up to 10 µg of RNA. Nucleotides, proteins, salts, and other impurities do not bind to the EZ-10 Column.

Applications

1. Preparation of Total RNA from various sources.
2. RT-PCR
3. Differential display
4. cDNA synthesis
5. PolyA + RNA selection
6. RNase/S1 nuclease protection

Features

√ Preparation of high quality total RNA from animal cells or other sources.
√ Rapid and Economical: entire procedure takes about 20 minutes (Note: timing for preparation of samples is not included).
√ High yield.
√ No phenol/chloroform extraction or ethanol precipitation needed.

NOTE: Care must be taken when working with RNA. It is important to maintain an RNAse-free environment starting with RNA sample preparation and continue through purification and analysis. Use RNAse free tubes, tips, gels. Wear gloves at all the time.

Procedures for Isolation of Total RNA from Animal Cells

1. Samples Preparation:

A. Harvest cells

a) Cells grow in suspension: Determine the number of cells. Spin down the appropriate number of cells for 5 minutes at 12,000 rpm (11,000 x g) in a RNase-free microtube. Carefully remove all supernatant by aspiration, and continue with Step B.

b) Cells grown in a monolayer. Determine the number of cells. Cells grow in a monolayer in cell-culture vessels can be trypsinized. Aspirate medium, and wash cells with PBS. Aspirate PBS and add 0.1-0.25% trypsin in PBS. After cells detach from the dish or flask, add medium (containing serum to inactivate the trypsin), transfer cells to a 1.5-ml RNaseA free microtube, centrifuge at 12,000 rpm (11,000 x g) for 5 minutes. Completely aspirate supernatant, and continue with Step B.

Note: Incomplete removal of the cell-culture will inhibit lysis and dilute the lysate, affecting the conditions for binding RNA to EZ-10 Spin Column. Both effects may reduce RNA yield.

B. Disrupt cells by addition of RLT Solution

a) For pelleted cells: loosen cell pellet by flicking the tube and then add RLT Solution.

b) For monolayer cells: add RLT Solution to monolayer cells (according to table below). Collect cell lysate and vortex. No cell clumps should be visible before proceeding to Step 2.

spin at 5,000 rpm (6,000 x g) for 1 minute. If blood is stored in RNA stabilizer, centrifuge the sample for 1 minute, thoroughly remove and discard the supernatant by aspiration or pouring, continue with step B.

b. Loosen cell pellet by flicking the tube and add 500 µl of RLT Solution.

2. Homogenize lysate: Two alternative methods may be used.
   (a) Homogenize for 30 seconds using a rotor-stator homogenizer.
   (b) Pass lysate at least 5 times through a 20-G (D=0.9mm) needle fitted to a syringe.
3. Add equal volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.
4. Place EZ Spin Column to 2.0-ml Collection Tube. Transfer above ethanol mixture to the column. Spin at 8,000 rpm (6,000 x g) for 1 minute.
   Note: precipitate may form after adding ethanol, but this will not affect the procedure.
5. Discard flow-through. Add 500 µl of RW Solution to the column and spin at 10,000 x rpm (8,000 x g) for 1 minute. Discard flow-through and place EZ Spin Column back to the same Collection Tube.
6. Add 500 µl of RPE Solution to the column, spin at 10,000 rpm (8,000 x g) for 1 minute. Discard flow-through and spin once more to remove residue of RPE Solution.
7. Transfer EZ Spin Column to a clean RNase-free 1.5 ml microtube. Add 30-50 µl of RNase-free water onto the center part of the column; incubate at 50℃ for 2 minutes. Spin down at 10,000 rpm (8,000 x g) for 1 minute. RNA is ready for use or kept at -70℃.

samples with high starch content, incubation is avoided to prevent swelling of starch material.

3. Add 330 µl of 100% ethanol to the mixture (step 2). Mix gently.
4. Place an EZ-10 Spin Column to 2.0-ml Collection Tube and transfer the mixture Step 3 to the column. Spin at 8,000 rpm (6,000 x g) for 1 minute. Discard the flow-through and place the column back to the same Collection Tube.
5. Add 500 µl of RW Solution to the column, centrifuge at 8,000rpm (6,000 x g) for 1 minute, and discard the flow-though.
6. Add 500 µl of RPE Solution and spin at 8,000 rpm (6,000 x g) for 1 minute. Discard the solution in the Collection Tube and spin once more to remove the residue of RPE Solution.
   Note: Ensure ethanol is added to RPE Solution before use.
7. Transfer the column to a clean RNase-free 1.5 ml microtube, add 30-50 µl of RNase-free H_2O onto the center part of the membrane in the column, and incubate at 50℃ for 2 minutes and spin down at 10,000 rpm (8,000 x g) for 1 minute. Keep the purified RNA sample at -70°C.

Note: To remove DNA from RNA, digest RNA from step 7 with DNASE I, RNASE FREE (Catalogue # DD0649). Follow procedures for RNA cleanup below to obtain DNA-FREE RNA.

Procedure for Isolation of RNA from Whole Blood

1. Sample preparation
   a. Add 1 ml treated water to 500 µl of fresh anticoagulated whole blood. Mix the sample by inverting the tube several times, and then

RLT Solution (µl)	Number of Pelleted Cells	Dish Diameter (cm)
350	> 5 x 10^6	6
600	> 5 x10^6 to 1 x 10^7	6-10

2. Homogenize lysate: two alternative methods may be used.
   a) Homogenize for 30 seconds using a rotor-stator homogenizer.
   b) Pass lysate at least 5 times through a 20-G (D=0.9mm) needle fitted to a syringe.
3. Add equal volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not centrifuge.
4. Place EZ-10 Spin Column into a 2.0-ml Collection Tube. Transfer above ethanol mixture to the column. Spin at 8,000 rpm (6,000 x g) for 1 minute.
   Note: precipitate may form after adding ethanol, but this will not affect the procedure.
5. Discard the flow-through. Add 500 µl of RW Solution to the EZ-10 Spin Column and spin at 8,000 rpm (6,000 x g) for 1 minute. Discard flow-through and place EZ-10 Spin Column back to the same Collection Tube.
6. Add 500 µl of RPE Solution to the EZ-10 Spin Column, spin at 8,000 rpm (6,000 x g) for 1 minute. Discard the flow-through and spin once more to remove residue of RPE Solution.
7. Transfer EZ-10 Spin Column to a clean RNase-free 1.5 ml microtube. Add 30-50 µl of RNase-free water onto the center part of the column; incubate at 50℃ for 2 minutes. Spin down at 10,000 rpm (8,000 x g) for 1 minute. RNA is ready for use or kept at – 70℃.

Note: To remove DNA from RNA, digest RNA from step 7 with DNASE I, RNASE FREE (Catalogue # DD0649). Follow procedures for RNA cleanup below to obtain DNA-FREE RNA.

Procedure for Isolation of Total RNA from Bacteria

1. Samples Preparation: Harvest the appropriate number of cells (<1x10^9) by centrifugation at 5000rpm (6,000 x g) for 3 minutes at 4℃. Discard supernatant, make sure all media is completely removed.
2. Add 100 µl of TE containing lysozyme and incubate. Gram-negative bacteria and Gram-positive bacteria require different amount of lysozyme and incubation time.

Bacteria	Concentration of Lysozyme in TE	Incubation Time
Gram-negative	400 µg / ml	3-5 minutes
Gram-positive	3 mg / ml	5-10minutes

(Note: Invert several times during incubation)

3. Add 350 µl of RLT Solution, mix vigorously. If insoluble material is visible, centrifuge for 2 minutes at 8,000 rpm (6,000 x g). Save the supernatant.
4. Add 260 µl of 100% ethanol to the supernatant, and mix gently. A precipitate may form by adding ethanol, but it will not affect the result.
5. Place an EZ-10 Spin Column to 2.0-ml Collection Tube and transfer the mixture from Step 4 to the column. Spin at 8,000 rpm (6,000 x g) for 1 minute. Discard flow-through and place the column back to the same collection tube.
6. Add 500 µl of RW Solution to the column, centrifuge at 8,000 rpm (6,000 x g) for 1 minute, and discard the flow-though.
7. Add 500 µl of RPE Solution and spin at 8,000 rpm (6,000 x g) for 1 minute. Discard the solution in the collection tube and spin once more to remove the residue of RPE Solution.
   Note: Ensure ethanol is added to RPE Solution before use.
8. Transfer the column to a clean RNase-free 1.5 ml microtube; add 30-50 µl of RNase-free H_2O onto the center part of the membrane in the column. And incubate at 50℃ for 2 minutes and spin down at 10,000 rpm (8,000 x g) for 1 minute. Keep purified RNA sample at -70℃.

Note: To remove DNA components within RNA, digest RNA from step 7 with DNASE I, RNASE FREE (Catalogue # DD0649). Follow procedures for RNA cleanup below to obtain DNA-FREE RNA.

Procedure for Isolation of Total RNA from Tissue, Plant Cells and Filamentous Fungi

1. Samples Preparation: Grind sample under liquid nitrogen to a fine powder using a mortar or pestle. Transfer the mixture of tissue powder and liquid nitrogen to 1.5 ml microtube and allow the liquid nitrogen to evaporate. Do not allow the sample to thaw. Proceed immediately to Step 2.
2. Add 450 µl of RLT Solution to a maximum of 100 mg of tissue powder and vortex vigorously. Incubation at 50℃ may help to disrupt the tissue sample. For