EZ-10 Spin Column Total RNA Minipreps Super Kit

SKU: BS583 | BS584 | BS784

Description	BS583 20 preps	BS584 100 preps	BS784 250 preps
RLT Solution[a]	14ml	70ml	175ml
RW Solution	12ml	60ml	150ml
RPE Solution[b]	5ml	22ml	2 x 30ml
RNAse-free Water	1ml	5ml	12.5ml
EZ-10 Spin Column & 2.0ml Collection Tube	20	100	250
Protocol	1	1	1

Notes

(a) RLT Solution should be ept at 2-8°C. It may forma precipitate upon storage. If necessary, dissolve the precipitate by warming the solution t 37°C.

(b) Before use:
BS583 - add 20 ml of 100% ethanol to 5 ml RPE Solution
BS584 - add 88 ml of 100% ethanol to 22 ml RPE Solution
BS784 - add120 ml of 100% ethanol to 30 ml RPE Solution

For other volumes of RPE Solution, simply add enough thanol to make a 4:1 ratio. (olume of added ethanol : volume of RPE Solution = 4:1)

Principle

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

Application

Preparation of otal RNA from various sources.

Features

- Preparation of high quality otal RNA from animal cells or other sources.
- Rapid and Economical: entie procedure takes about 20 minutes (Note: timing or preparaation of samples isnot included).
- High yield.
- No phenol/chloroform extraction or thanol precipitaati onneeded.

NOTE: Care must be taken when working with RNA. It is important to maintain an RNAse-free environment startin gwithRNA sample preparaation andonontinue through purificaation and anasis. Use RNAse frfree tubes, tipsels. 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 Sep B.

b) 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. Afer cells detach from the dish or flask, add medium (containing serum to inacti ate 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 wit hSep B.

NOTE: Incomplete removal of the cell-culture will inhibit lysis and dilute the lysate, afffecting the conditionor 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 o monolayer cells (according to table below). Collect cell lysate and vortex. No cell clumps should be visible before proceeding to Step 2.

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

2. **Homogenize lysate: two alternati e 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 f ed to a syringe.
3. Add equal volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not c trifuge.
4. Place EZ-10 Spin Column into a 2.0-ml Collection ube. Transfer above ethanol mixture to the column. Spin at 8,000 rpm (6,000 x g) for 1 minute.

NOTE: Precipitate may form afer adding ethanol, but this will not affect the procedure.

5. Discard the flow-through. Add 500 µl of RRW Solutiono 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 SpinColumn back to the same Collection ube.
6. Add 500 µl of RPE Solution o 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 5°C 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°C.

NOTE: To remove DNA from RNA, digest RNA from step 7 with DNASE I, RNASE FREE (Catalogue # Dd0649). Follow procedures for RNA cleanup on the following page to obtain DNA-FREE RNA.

Procedures for Isolation of Total RNA from Bacteria

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

Bacteria	Concentration of Lysozyme in TE	IncubationTime
Gram-negati e	400 µg / ml	3-5 minutes
Gram-positi e	3 mg / ml	5-10 minutes

(**NOTE:** Invert several times during incub ation)

3. Add 350 ul of RLT Solution, mix vi orously. If insoluble material is visible, centrifuge for 2 minutes at 8,000 rpm (6,000 x g). Save the supernatant.

4. Add 260 ul 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 ube 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 ccollection tube.
6. Add 500 ul of RW Solution o the column, centrifuge at 8,000 rpm (6,000 x g) for 1 minute, and discard the flow-though.
7. Add 500 ul of RPE Solution and spin t 8,000 rpm (6,000 x g) for 1 minute. Discard the solution in the collection tube and spin once m e to remove the residue of RPE Solution.

NOTE: Ensure ethanol is added to RPE Solution b fore use.

8. Transfer the column to a clean RNase-free 1.5 ml microtube; add 30-50 ul of RNase-free H2O onto the center part of the membrane in the column. And incubate at 50°C for 2 minutes and spin down at 10,000 rpm (8,000 x g) for 1 minute. Keep purified RNA sample at -70°C.

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.

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

1. Samples Preparation: Grind sample under liquid nitogen to a fine powder using a mortar or pestle. Transfer the mixture of tissue po der 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 ul of RLT Solution o a maaximum of 100 mg of tissue p der and vortex vigorously. Incubation t 50°C may help tto disrupt the tissue sample. For samples with high starch content, incubation is voided to prevent swelling of starch material.
3. Add 330 ul of 100% ethanol to the mixture (step 2). Mix gently.
4. Place an EZ-10 Spin Column to 2.0-ml Collection ube 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 tto the same Collection Tube.
5. Add 500 ul of RW Solution o the column, centrifuge at 8,000rpm (6,000 x g) for 1 minute, and discard the flow-through.

6. Add 500 ul of RPE Solution and spin t 8,000 rpm (6,000 x g) for 1 minute. Discard the solution in the Collection ube and spin once more to remove the residue of RPE Solution.

NOTE: Ensure ethanol is added to RPE Solution b fore use.

7. Transfer the column to a clean RNase-free 1.5 ml microtube, add 30-50 ul of RNase-free H2O onto the center part of the membrane in the column, and incubate at 50°C 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.

Procedures for Isolation of RNA from Whole Blood

1. **Samples Preparation:**

   a) Add 1 ml treated water to 500 µl of fresh anti oagulated whole blood. Mix the sample by inverting the tube se erral times, and then spint 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 pourin , conntinue withtep b.
   b) Loosen cell pellet by flicking the tube and add 500 µl of RLLT Solution.

2. **Homogenize lysate: Two alternati e 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 f ed to a syringe.

3. Add equal volume of 70% ethanol to the homogenized lysate, and mix well by pipetting. Do not c trifuge.

4. Place EZ Spin Column to 2.0-ml Collection ube. Transfer above ethanol mixture to the column. Spin at 8,000 rpm (6,000 x g) for 1 minute.

NOTE: Precipitate may form afer adding ethanol, but this will not affect the procedure.

5. Discard flow-through. Add 500 µl of RRW Solutiono 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 ube.

6. Add 500 µl of RPE Solution o 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°C 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°C.

NOTE: To remove DNA from RNA, digest RNA from step 7 with DNASE I, RNASE FREE (Catalogue #Dd0649). Follow procedures for RNA cleanup on the following page to obtain DNA-FREE RNA.

Procedures for RNA Cleanup

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

1. Adjust sample to a volume of 100 µl with RNase-free H2O, add 350 µl of RLT Solution, and mix ell. 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 ube and transfer the mixture solution (Sep 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 o 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 o 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 H2O 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°C.

Storage

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

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NOT INTENDED FOR HUMAN OR ANIMAL USE