PRODUCT INFORMATION

96-Well Plate Bacterial Total RNA Mini-Preps Super Kit

Product information for BS5852/BS585:

Component:

Component	BS5852, 2 plates	BS585, 5 plates
Buffer Rlysis-BG	80 ml	200 ml
Universal GT Solution	72 ml	2 X 90 ml
Universal NT Solution	24 ml	60 ml
RNase-free Water	10 ml	25 ml
EZ-10 96-Well Plate	2	5
Deep Well Collection Plate	4	10
96-well Storage Plate	2	5
Sealing Film	6	15
Protocol	1	1

Storage:

Transport at room temperature, store all components at 4^{o}C upon received.

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Introduction:

This kit provides a simple and fast extraction of high quality of total RNA from a wide range of biological samples including Gram negative and positive bacteria using a high throughput spin-column format. The total RNA in the lysate of bacteria is selectively bound on each column of the 96-well plate and other impurities such as proteins and salts do not bind. The entire procedure takes approx 15 minutes only. No ethanol precipitation is required. Purified bacterial total RNA can be used for many downstream applications such as RT-PCR，Northern Blot，cDNA synthesis. The kit is also suitable for RNA extraction from animal, fungi and some of plants samples. The kit may not be suitable for RNA extraction from the plants containing high levels of secondary metabolites, polyphenols and polysaccharides. For these plants, Plant RNA Isolation Kit and EZ-10 Spin Column Plant RNA Isolation Kit are recommended (Codes PT4191 and BS82314).

Feature:

1. Fast. Using a rapid spin and 96-well high throughput format, the entire procedure takes less than 15 minutes only.
2. High Purity of RNA. Purified RNA has an OD_{260}/OD_{280} ratio of 1.8-2.0.
3. Suitable for RNA extraction from both of Gram negative and positive bacteria.
4. Economic.

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 times.

Universal GT Solution and Universal NT Solution are supplied in a concentrated form, before use, add 48 ml

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and 60 ml of 96-100% ethanol to 72 ml and 90 ml concentrated universal GT solution respectively. Add 96 ml and 240ml of 96-100% ethanol to 24 ml and 60 ml of concentrated universal NT solution to make a work solution.

Procedure:

1. Sample Preparation.
2. A. Gram-negative bacterium:
   a. Transfer 1 ml logarithmic phase culture (about 2 x 10^9 cells) into Deep Collection Plate and centrifuge at 5,000 *x g* for 1 min, discard supernatant.
   b. Add 100 µl lysozyme solution (400µg/ml lysozyme in RNase-free water. NOT supplied in the kit), suspend thoroughly and incubate at 37°C for 5 minutes.

   B. Gram-positive bacterium:
   a. Transfer 1 ml logarithmic phase culture (about 2 x 10^9 cells) into Deep Collection Plate and centrifuge at 5,000 *x g* for 30 seconds, discard supernatant.
   b. Add 100 µl lysozyme solution (3mg/ml lysozyme in RNase-free water. NOT supplied in the kit), suspend thoroughly and incubate at 37°C for 5 minutes.
3. Add 350 µl Buffer RIysis-BG and seal the Deep Well Collection Plate using a Sealing Film, mix by inverting immediately.
4. Add 1/2 volume of ethanol, close with Sealing Film and mix thoroughly.

Vacuum Based Procedure

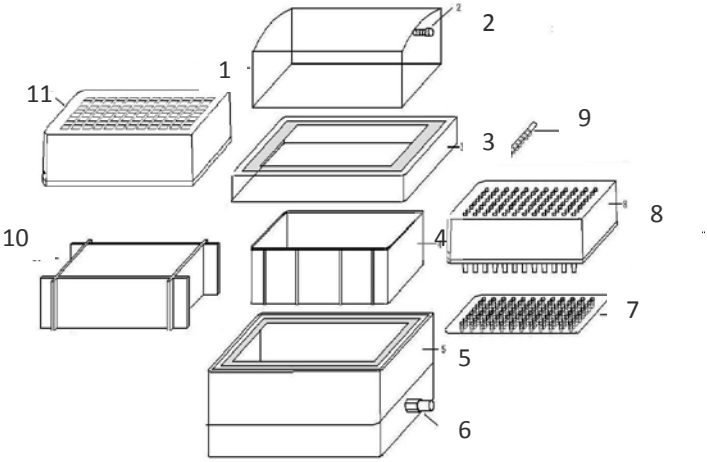


Figure 1. *Components of EZ-10 96 Well Spin Column Plasmid DNA Minipreps Kit*

1. Top Cap
2. Release Valve
3. Base Cap
4. Waste Tray
5. Base
6. Vacuum Connector
7. 96 Well Storage Plate
8. 96 Well Filter Plate (blue nozzle) or 96 Well Plate (pink nozzle)
9. 8 Well Strip Vacuum Sealer
10. 96 Well Storage Plate Holder
11. Deep Well Collection Plate

Note: Vacuum Manifold (SD5011 – including 1, 3, 4, 5, 10) and 8 Well Strip Vacuum Sealer (BP547) are sold separately.

5. Assemble the vacuum manifold. Place a Waste Tray in the Base, cover it with the Base Cap, and put a clean EZ-10 96 Well Plate (pink nozzle) on top.

6. Pipet the lysates carefully from step 5 into the wells of an EZ-10 96 Well Plate. Apply vacuum until all samples have passed through.

7. Switch off the vacuum and ventilate the vacuum manifold slowly. Add 500 µl of Universal GT Solution to each well and apply vacuum until buffer has passed through.

8. Add 500 µl of Universal NT Solution to each well and apply vacuum until buffer has passed through.

9. After Wash Solution has been drawn through all wells, apply maximum vacuum for an additional minute to dry the membrane.

10. Switch off the vacuum and ventilate the vacuum manifold slowly. Remove the EZ-10 96 Well Plate together with the Base Cap from the Base. Vigorously tap the plate on a stack of absorbent paper, and blot the nozzles of the EZ-10 Well Plate with clean adsorbent paper until no droplets remain.

11. For elution, assemble the vacuum manifold. Place the Storage Plate Holder in the Base, put 96 Well Storage Plate on top, and cover it with Base Cap. Place EZ-10 96 Well Plate on top securely. Mark the orientation appropriately.

12. To elute, pipet 50 µl RNase-free Water onto the center of each well of the EZ-10 96 Well Plate, incubate for 1 minute, and apply vacuum (–550 to –650 mbar) for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.

13. Tightly seal the 96 Well Storage Plate. RNA is ready for use or store at -80°C freezer.

Note: It is important to add the Elution Buffer into the center of each well.

Centrifugation Based Procedures

14. Place the EZ-10 96-Well Plate to a new Deep Well Collection Plate. Transfer the solution to the EZ-10 96-Well Plate and centrifuge at 5,000× *g* for 2 min at room temperature. Discard the flow through.
15. Place the EZ-10 96-Well Plate into a Deep Well Collection Plate. Add 0.5 ml of Universal GT Solution to the EZ-10 96-Well Plate, and centrifuge at 5,000 × *g* for 1 min at room temperature. Discard the flow through.
16. Place the EZ-10 96-Well Plate back to a Deep Well Collection Plate. Add 0.5 ml of Universal NT Solution to the EZ-10 96-Well Plate, and centrifuge at 5,000 × *g* for 1 min at room temperature. Discard the flow through.
17. Place the EZ-10 96-Well Plate to a Deep Well Collection Plate, centrifuge the column at 5,000× *g* for 2 min at room temperature.

    Note: This step is very important to remove the residual ethanol thoroughly.

18. Place the EZ-10 96-Well Plate to a Deep Well Storage Plate, add 50 µl RNase-free Water, and keep at room temperature for 2 minutes. Centrifuge at 5,000 × *g* for 30 sec at room temperature, save the eluted RNA solution at -80°C.

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Other Kits Available

EZ-10 Spin Column Plasmid DNA MiniPreps Kit
BS413, BS414

EZ-10 Spin Column PCR Products Purification Kit
BS363, BS364

EZ-10 Spin Column DNA Gel Extraction Kit
BS353, BS354

EZ-10 96-Well Spin Column PCR Products Purification Kit
BS3652 (2preps), BS365 (5preps)

And much more……..

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PRODUCTS ARE INTENDED FOR BASIC SCIENTIFIC RESEARCH ONLY!
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