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BRC1251
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CV2 2020

For Research Use Only

EZ-10 96 Well Plate RNA Cleanup & Concentration Kit

Code: BRC1251 (2 Plates)

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Description

EZ-10 Spin Column RNA Cleanup & Concentration kit is designed for rapid purification and concentration from in vitro transcription products or total RNA isolated from various methods such as labeling or DNase digestion.

Features

- ✓ Recovery of RNA larger than 20nt.
- ✓ No toxic organic chemicals used.
- ✓ Rapid and convenient, the whole procedure takes only 5 minutes.
- ✓ Rate of recovery higher than 80%.
- ✓ Compatible with most downstream applications.

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.

Storage

Transportation at room temperature. Upon receipt, store all components at 4°C. The kit is stable for up to 12 months at 4°C.

7. To elute, place a 96 Well Storage Plate on top of a Deep Well Collection Plate, and then place the EZ-10 96 Well Binding Plate on the top of a 96 Well Storage Plate. Add 30-100 µl of RNase-free Water into the center part of the membrane of each well and incubate at 37-50°C for 2 minutes. Spin at 4,500 xg for 5 minutes.

NOTE: 96 Well Storage Plate is very fragile and needs to be placed on top of a Deep Well Collection Plate for support during centrifugation.

8. RNA is ready for use or store at -80°C freezer.



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10. To elute RNA, pipette 30-100 μ l RNase-free Water onto the center of each well of the EZ-10 96 Well Binding Plate, incubate for 1 minute, and apply vacuum (-550 to -650 mbar) for 1 minute. Switch off vacuum and ventilate vacuum manifold slowly.
11. 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

NOTE: For centrifugation based method, there is a minimum height requirement of 75 mm for apparatus to hold the assembly of EZ-10 96 Well Spin Column Plate and Deep Well Collection Plate.

3. Place an EZ-10 96 Well Binding Plate on top of a new Deep Well Collection Plate. Transfer the above supernatants (clear lysate) from step 2 into EZ-10 96 Well Binding Plate using an 8-channel pipette. Centrifuge at $5,700 \times g$ for 5 minutes.

NOTE: If the total volume exceeds $700 \mu\text{l}$, repeat this step until all solution pass through the column. RNA columns do not have DNase on-column. In order to remove DNA, Dnase digestion is required (not supplied in the kit).

4. Discard the flow-through in the Deep Well Collection Plate. Add $500 \mu\text{l}$ of RPE Wash Solution to each well of EZ-10 96 Well Binding Plate, and centrifuge at $5,700 \times g$ for 5 minutes.

NOTE: Universal RPE Solution is supplied as a concentration. Ensure that ethanol is added to Universal RPE Solution before use.

5. Repeat step 4. (Optional: Repeat wash step one more time if needed)
6. Discard the flow-through in the Deep Well Collection Plate. Spin at $5,700 \times g$ for additional 5 minutes to remove residual Wash Solution.

Contents

Components	BRC1251 (2 Plates)
Buffer RLT	200 ml
Universal RPE Solution	2x24 ml
RNase-free Water	20 ml
EZ-10 96 Well Binding Plate	2
2ml Deep Well Collection Plate	4
96 Well Storage Plate	2
Sealing Film	8
Protocol	1

* Before use, add 96 ml of 100% ethanol (RNase free) to 24 ml Universal RPE Solution.

Starting Material

A maximum of $100 \mu\text{g}$ RNA can be used in the RNA cleanup protocol. This amount corresponds to the binding capacity of the RNeasy mini columns.

Important Notes

We recommend DNase digestion or kit BS88133 to prepare DNA-free RNA.

Procedure

1. Add 9 volumes of Buffer RLT to RNA solution, mix thoroughly by pipetting.

NOTE: Do not centrifuge.

2. Add 1/2 volume of ethanol, mix by inverting the tube.

Vacuum Based Procedure

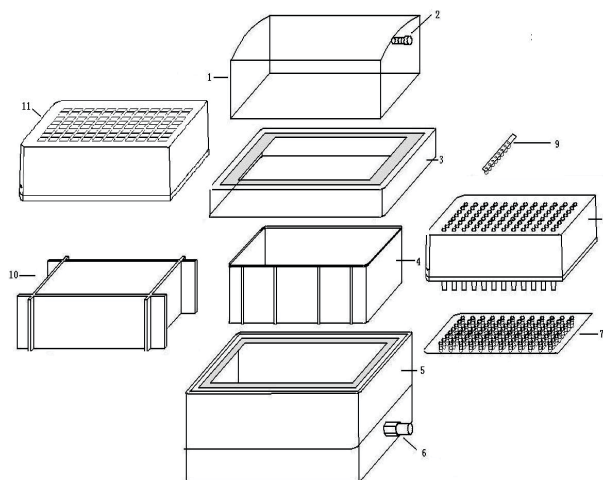


Figure 1. Components of EZ-10 96 Well Spin Column Kit

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

NOTE: Vacuum Manifold (SD5011 – including 1, 3, 4, 5, 10) is sold separately.

3. 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 Binding Plate (pink nozzle) on top.
4. Pipette the mixture from step 2 carefully into the wells of EZ-10 96 Well Binding Plate. Apply vacuum until all samples have passed through. Do not pipette any debris into the 96 Well Plate as they may clog the wells.

NOTE: If the total volume exceeds 700 μ l, repeat this step until all solution pass through the column. RNA columns do not have DNase on-column. In order to remove DNA, DNase digestion is required (not supplied in the kit).

5. Switch off the vacuum and ventilate the vacuum manifold slowly. Add 500 μ l Universal RPE Solution to each well and apply vacuum until buffer has passed through.

NOTE: Universal RPE Solution is supplied as a concentration. Ensure that ethanol is added to Universal RPE Solution before use.

6. Repeat step 5. (Optional: Repeat wash step one more time if needed).
7. After Wash Solution has been drawn through all wells, apply maximum vacuum for an additional minute to dry the membrane.
8. Switch off the vacuum and ventilate the vacuum manifold slowly. Remove the EZ-10 96 Well Binding 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 Binding Plate with clean adsorbent paper until no droplets remain.
9. 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 Binding Plate on top securely. Mark the orientation appropriately.