SV 96 Total RNA Isolation System

INSTRUCTIONS FOR USE OF PRODUCTS Z3500 AND Z3505.

Quick PROTOCOL

RNA Isolation from Cells Seeded in 96-Well Plates

Production of Sample Lysate and Binding of RNA to the Binding Plate

1. Wash cells once with 1X PBS.
2. Add 100µl of RNA Lysis Buffer to each well; mix by pipetting.
3. Transfer the cell lysate to a well of the SV 96 Binding Plate positioned in the vacuum Manifold Base. (See reverse for set-up of the Vac-Man® 96 Vacuum Manifold.)
4. Apply vacuum until solution passes through the plate. Release vacuum.

Wash

1. Add 500µl of RNA Wash Solution to each well.
2. Apply vacuum until solution passes through the plate. Release vacuum.

DNase Treatment and Wash

1. Prepare DNase incubation mix using the chart below:

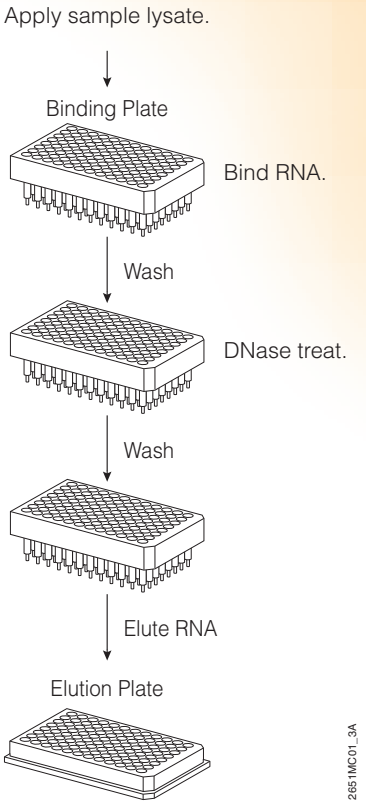
Solution	Volume Per Prep	×	Number of Preps	=	Total
Yellow Core Buffer	20µl				
$MnCl_2$	2.5µl				
DNase I	2.5µl				

Mix by gentle pipetting; do not vortex.

2. Add 25µl of DNase incubation mix to each well of the SV 96 Binding Plate. Incubate at room temperature for 10 minutes.
3. Add 200µl of DNase Stop Solution to each well of the SV 96 Binding Plate.
4. Apply vacuum until solution passes through the plate. Release vacuum.
5. Add 500µl of RNA Wash Solution.
6. Apply vacuum until solution passes through the plate.
7. Apply vacuum for an additional 10 minutes to allow binding matrix to dry. Release vacuum.

Elution

1. Position the Binding Plate on the Manifold Collar above the Elution Plate. Add 100µl Nuclease-Free Water to each well of the Binding Plate; incubate for 1 minute. Apply vacuum for 1 minute.
2. Release the vacuum and remove the Binding Plate and Manifold Collar. If droplets are on the sides of the Elution Plate wells, centrifuge the plate to collect drops on the well bottoms. Store samples at –20°C or –70°C after covering with Plate Sealer.



See additional protocol information in Technical Bulletin #TB294, available online at **www.promega.com**

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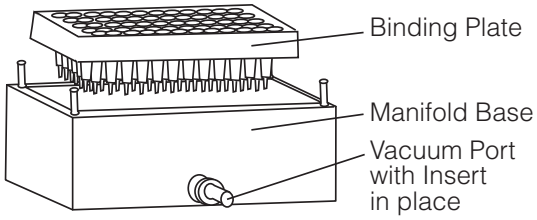
SV 96 Total RNA Isolation System

INSTRUCTIONS FOR USE OF PRODUCTS Z3500 AND Z3505.

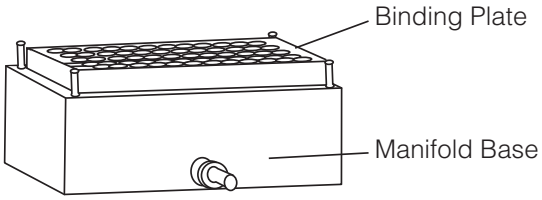
Quick PROTOCOL

Set-Up of the Vac-Man® 96 Vacuum Manifold

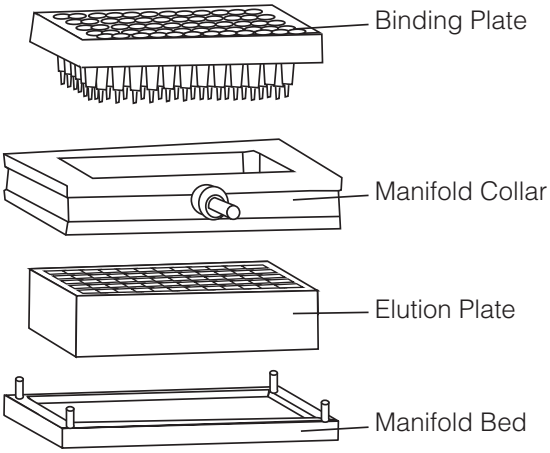
A. Total RNA Binding Apparatus



B. Washing Apparatus



C. Elution Apparatus



The Vac-Man® 96 Vacuum Manifold with the SV 96 Total RNA Isolation System components. Panels A, B and C show the manifold and plate combinations necessary to accomplish RNA binding, washing and elution, respectively, for manual total RNA purification. For automated total RNA purification on the Beckman Coulter Biomek® 2000, the Beckman vacuum manifold (Beckman Coulter Part# 609670) and 36mm vacuum collar (Beckman Coulter Part# 609597) are required. See Automated Protocol #EP003 (available online at **www.promega.com/tbs/**) for additional information regarding automated RNA purification.

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