SV Total RNA Isolation System

INSTRUCTIONS FOR USE OF PRODUCTS Z3100, Z3101 AND Z3105.

Quick PROTOCOL

Spin Protocol

1. Place **175µl RNA Lysis Buffer (RLA)** (+ BME) in an autoclaved tube.
2. Prepare sample for lysis.
3. Immediately place sample into **Lysis Buffer**. Mix thoroughly by inversion. **Note:** Ensure proper ratio of Lysis Buffer to sample. See Table 1 of the standard protocol.*
4. Add **350µl RNA Dilution Buffer** (RDA, blue). Mix by inverting 3–4 times. **Note:** Refer to the appropriate lysate preparation section in the Technical Manual #TM048 to determine whether the sample should be heated at 70°C for 3 minutes.
5. Centrifuge for 10 minutes. Transfer the cleared lysate to a fresh tube.
6. Add **200µl 95% ethanol** to cleared lysate and mix well (pipet).

The Spin and Vacuum Protocols are identical up to this point.

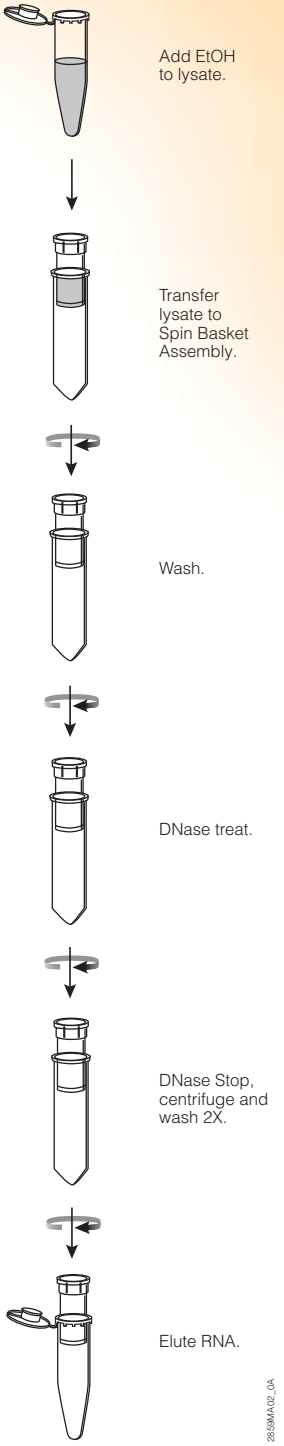
7. Transfer mixture to Spin Basket Assembly and centrifuge for 1 minute. Discard eluate.
8. Add **600µl** of **RNA Wash Solution (RWA)** (+ ethanol). Centrifuge for 1 minute and discard the eluate.
9. Prepare **DNase incubation mix** using the table below:

Solution	Volume	×	Number of Preps	=	Total
Yellow Core Buffer	40µl				
$MnCl_2$, 0.09M	5µl				
DNase I	5µl				

*Mix gently (pipet); do **not** vortex.*

10. Apply **50µl** of DNase mix to membrane. Incubate at RT for 15 minutes.
11. Add **200µl DNase Stop Solution (DSA)** (+ ethanol) and centrifuge for 1 minute.
12. Add **600µl RNA Wash Solution (RWA)**; centrifuge for 1 minute. Empty.
13. Add **250µl RNA Wash Solution (RWA)**; centrifuge for 2 minutes. Transfer Spin Basket to Elution Tube.
14. Add **100µl Nuclease-Free Water** to membrane. Centrifuge for 1 minute to elute the RNA and store at –70°C.

RT: room temperature
Centrifugation: 12,000–14,000 × *g* (at RT)



*Additional protocol information is available in Technical Manual #TM048, available online at: **www.promega.com**

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Quick PROTOCOL

Vacuum Protocol

Note: *For the Vacuum Protocol, follow Steps 1–6 of the Spin Protocol.*

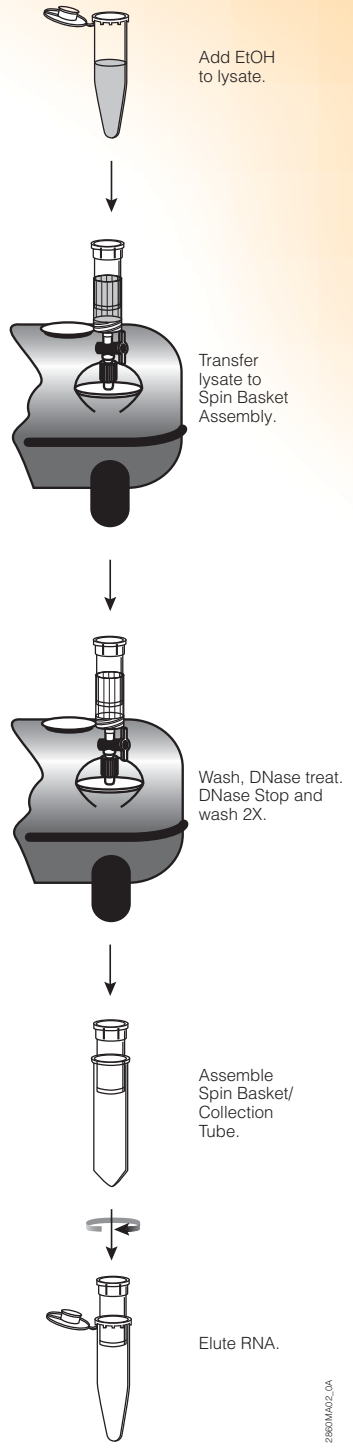
7. Attach Vacuum Adapter with Luer-Lok® fitting to one manifold port. Gently press SV RNA Spin Basket into adapter and transfer mixture to Spin Basket. Apply vacuum. **Note:** Label Collection Tube and save for Step 13.
8. Add **900µl RNA Wash Solution (RWA)**. Apply vacuum until solution has passed through. Stop vacuum source and open unused port to vent manifold. *Release all vacuum pressure before continuing!*
9. Prepare **DNase incubation mix** using the table below:

Solution	Volume	×	Number of Preps	=	Total
Yellow Core Buffer	40µl				
$MnCl_2$, 0.09M	5µl				
DNase I	5µl				

*Mix gently (pipet); do **not** vortex.*

10. Apply **50µl** of DNase incubation mix to membrane. Incubate at RT for 15 minutes.
11. Add **200µl DNase Stop Solution (DSA)** (+ ethanol) to Spin Basket. Close open port and apply vacuum.
12. Add **900µl RNA Wash Solution (RWA)**. Repeat wash.
13. Release vacuum pressure. Place Spin Basket in Collection Tube (from Step 7). Centrifuge Spin Basket/Collection Tube for 1 minute.
14. Transfer Spin Basket to Elution Tube, add **100µl Nuclease-Free Water** and centrifuge for 1 minute. Store purified RNA at –70°C.

RT: room temperature
Centrifugation: 12,000–14,000 × *g* (at RT)



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