

PolyATtract® System 1000

INSTRUCTIONS FOR USE OF PRODUCTS Z5400 AND Z5420.
FOR LABORATORY USE.

Quick
PROTOCOL

mRNA Isolation from Tissues

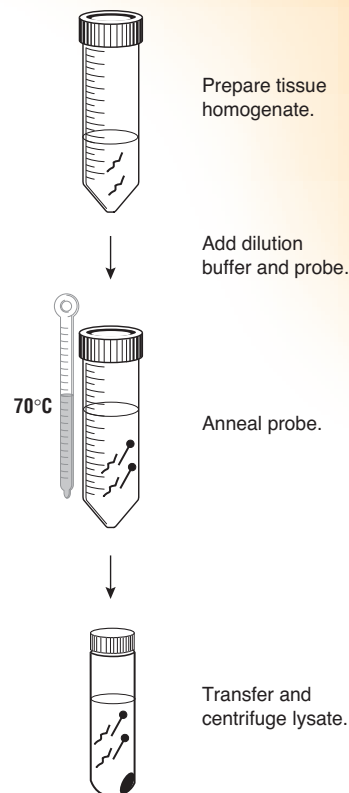
Sample Preparation

Use Table 2 and Figure 3 (5–100mg of tissue; Section 4.A of TM228) or Table 3 and Figure 4 (125–1,000mg of tissue; Section 4.B of TM228) to determine the quantity of reagents and SA-PMPs to use for various tissue sample sizes.

1. Warm the GTC Extraction Buffer, Biotinylated Oligo(dT) Probe, Nuclease-Free Water and SSC 0.5X Solution to room temperature. Preheat the Dilution Buffer to 70°C.
2. Add 41µl of β-Mercaptoethanol (48.7%) per milliliter of Extraction Buffer (Extraction/BME Buffer). Final concentration of BME is 2%.
3. Weigh the tube containing the buffer, and record the weight.
4. Place tissue in Extraction/BME Buffer, and homogenize. Weigh the tube containing the tissue in Extraction/BME Buffer. Calculate the tissue mass by subtracting the weight obtained in Step 3 from this new weight.

Probe Annealing

5. Refer to Section 4.A or 4.B of TM228 to determine the amount of Biotinylated Oligo(dT) Probe and SA-PMPs that are necessary for the tissue mass calculated in Step 4.
6. Dispense the preheated Dilution Buffer to a sterile tube, and add 20.5µl of β-Mercaptoethanol (48.7%) per milliliter of Dilution Buffer. Add Dilution Buffer/β-Mercaptoethanol mixture to the homogenate, and mix thoroughly by inversion.
7. Add the amount of Oligo(dT) Probe determined in Step 5, and mix well by shaking. Incubate this mixture at 70°C for 5 minutes.
8. Transfer the lysate to a clean, sterile 15ml centrifuge tube. Centrifuge at 12,000 × *g* for 10 minutes at **room temperature** to clear the homogenate.
9. Proceed to Washing Streptavidin Paramagnetic Particles.



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mRNA Isolation from Cell Cultures

Sample Preparation

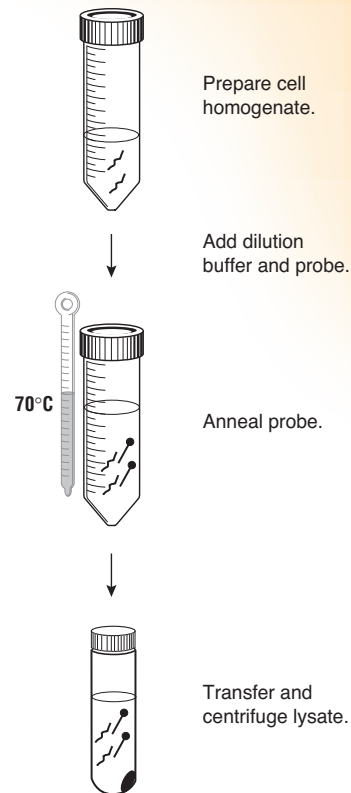
Refer to Table 4 (Section 4.D of TM228) for appropriate reagent and SA-PMP quantities based on the starting number of cells.

1. Warm the GTC Extraction Buffer, Biotinylated Oligo(dT) Probe, Nuclease-Free Water and SSC 0.5X Solution to room temperature. Preheat the Dilution Buffer to 70°C.
2. Add 41µl of β-Mercaptoethanol (48.7%) per milliliter of Extraction Buffer (Extraction/BME Buffer).
3. Collect 1×10^6 – 1×10^8 cells in a sterile conical tube by centrifugation at $300 \times g$ for 5 minutes. Wash the cell pellet with 25ml of ice-cold, sterile 1X PBS, and centrifuge at $300 \times g$ for 5 minutes to collect the cells. Pour off the supernatant.
4. Add the Extraction/BME Buffer to the cells. Homogenize the cells.

Probe Annealing

5. Dispense the preheated Dilution Buffer to a sterile tube, and add 20.5µl of β-Mercaptoethanol (48.7%) per milliliter of Dilution Buffer. Add Dilution Buffer/β-Mercaptoethanol to the homogenate, and mix thoroughly by inversion.
6. Add the Biotinylated Oligo(dT) Probe, and mix well. Incubate this mixture at 70°C for 5 minutes.
7. Transfer the lysate to a clean, sterile 15ml centrifuge tube. Centrifuge at $12,000 \times g$ for 10 minutes at **room temperature** to clear the homogenate.
8. Proceed to Washing Streptavidin Paramagnetic Particles.

See additional protocol information in Technical Manual #TM228, available online at: www.promega.com



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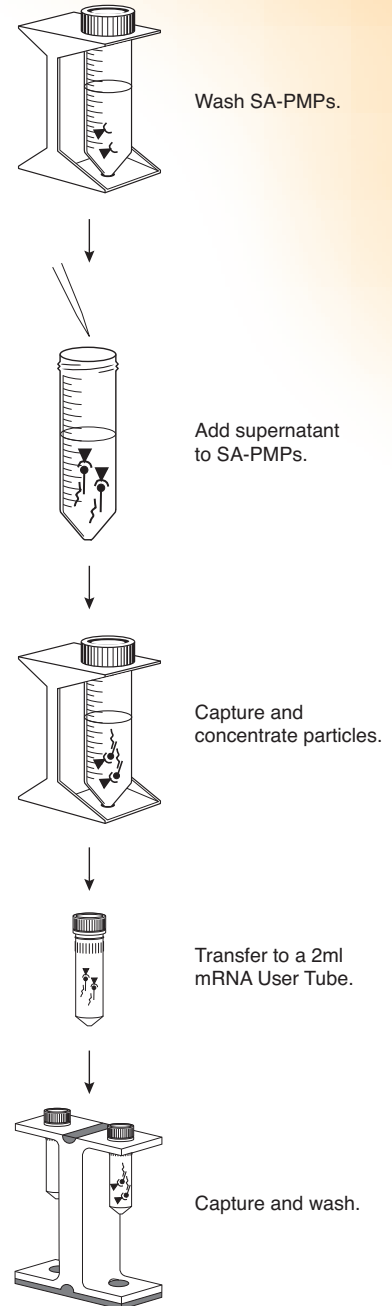
Quick
PROTOCOL

Washing Streptavidin Paramagnetic Particles (SA-PMPs)

1. Completely resuspend SA-PMPs by gently rocking the bottle.
2. Transfer SA-PMPs to a sterile conical tube **away from** the Magnetic Stand. Place the tube on the Magnetic Stand. (With 15ml tubes, use the Adapter.) **Slowly** move the Magnetic Stand toward the horizontal position until the particles are collected at the side of the tube. Carefully pour off the storage buffer.
3. Resuspend SA-PMPs in 0.5X SSC to the original volume. Capture the particles using the Magnetic Stand. Pour off the SSC.
4. Repeat this wash step twice more for a total of three times. Resuspend to the original volume with 0.5X SSC. **Do not** centrifuge the particles.

Capture and Washing

1. When centrifugation of the homogenate is complete, carefully transfer the supernatant to the tube containing the washed SA-PMPs in 0.5X SSC **away from the Magnetic Stand**. Mix by inversion.
2. Incubate the homogenate/SA-PMP mixture at room temperature for 2 minutes.
3. Capture SA-PMPs by using the Magnetic Stand. Move the Magnetic Stand toward the horizontal position until the homogenate clears. Carefully pour off the supernatant, and save in a sterile tube on ice.
4. Resuspend the particles in 0.5X SSC (to the volume indicated in Table 2 or 3 of TM228) by gently flicking the tube **away from the Magnetic Stand**. Transfer the particle mixture to a 2ml mRNA User Tube. Capture the particles by placing the tube on the Magnetic Stand. Carefully remove the SSC solution with a pipette.
5. Repeat this wash step twice. After the final wash, remove as much of the SSC solution as possible without disturbing the SA-PMPs.
6. Proceed to Elution of mRNA.



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Elution of mRNA

1. Add the amount of Nuclease-Free Water indicated in Table 2, 3 or 4 of TM228 to the SA-PMPs. Gently resuspend the particles by flicking the tube.
2. Magnetically capture SA-PMPs as before. Transfer the liquid containing the eluted mRNA to a sterile, RNase-free microcentrifuge tube, and save on ice.

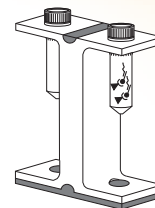
Note: If any particles were transferred, microcentrifuge the supernatant at $12,000 \times g$ for 1 minute. Transfer the supernatant to a fresh tube, and immediately place on ice.

Precipitation and Concentration of mRNA

1. **For cDNA cloning:** Add 0.1 volume of 3M sodium acetate (pH 5.2) and 1.0 volume of isopropanol to the eluate, and incubate at -20°C overnight.
For in vitro translation: Add 0.1 volume of 3M potassium acetate and 1.0 volume of isopropanol to the eluate, and incubate at -20°C overnight.
2. Centrifuge at $>12,000 \times g$ for 10 minutes. Resuspend the RNA pellet in 1ml of 70% ethanol, and centrifuge again.
3. **For short-term storage (<30 days):** Dry the pellet in a vacuum desiccator for about 15 minutes, resuspend in RNase-free, deionized water at 0.5–1.0mg/ml and store at -70°C .

For long-term storage (≥ 30 days): Store the RNA pellet in 70% ethanol at -70°C .

See additional protocol information in Technical Manual #TM228, available online at: www.promega.com



Capture particles.



Elute mRNA.



Precipitate and concentrate mRNA.

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