PolyATtract® System 1000

INSTRUCTIONS FOR USE OF PRODUCTS Z5400 AND Z5420.

Quick PROTOCOL

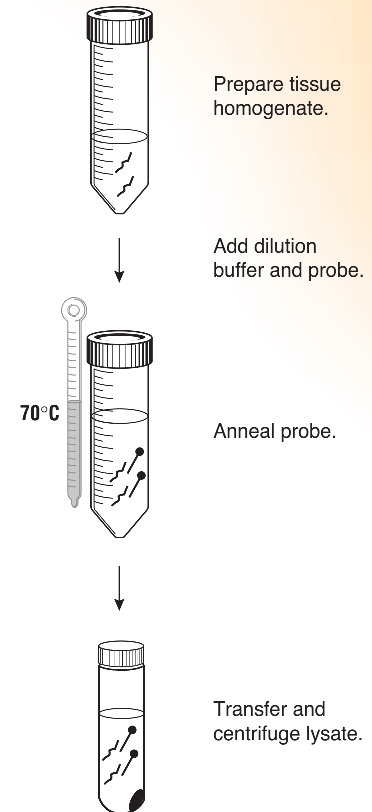
mRNA Isolation from Tissues

(*For cell culture protocol, see reverse.*)

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.

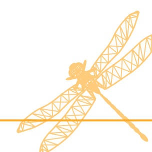
1. Warm the GTC Extraction Buffer, Biotinylated Oligo(dT) Probe, Nuclease-Free Water and SSC 0.5X Solution to room temperature. Preheat the Dilution Buffer to 70°C.
2. Add 20.5µl of β-Mercaptoethanol (97.4%) per milliliter of Extraction Buffer (Extraction/BME Buffer). Final concentration of BME is 2%.
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. Aliquot the preheated Dilution Buffer to a sterile tube and add 10.25µl of β-Mercaptoethanol (97.4%) per milliliter of Dilution Buffer. Add Dilution Buffer/β-Mercaptoethanol mixture to the homogenate and mix thoroughly by inversion.
7. Add the amount of 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.

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601



Promega

© 2000–2016 Promega Corporation. All Rights Reserved.

Printed in USA. Revised 8/16
Part #9FB047

PolyATtract® System 1000

INSTRUCTIONS FOR USE OF PRODUCTS Z5400 AND Z5420.

Quick PROTOCOL

mRNA Isolation from Cell Cultures

(*For tissue protocol, see reverse.*)

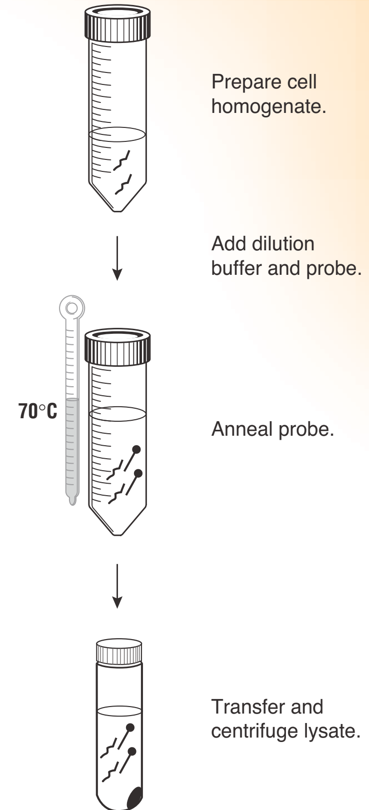
Sample Preparation

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

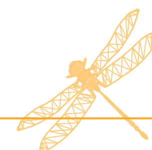
1. Warm the GTC Extraction Buffer, Biotinylated Oligo(dT) Probe, Nuclease-Free Water and SSC 0.5X Solution to room temperature. Preheat the Dilution Buffer to 70°C.
2. Add 20.5µl of β-Mercaptoethanol (97.4%) per milliliter of Extraction Buffer (Extraction/BME Buffer).
3. Collect 1×10^6–1×10^8 cells in a sterile conical tube by centrifugation at 300 × *g* for 5 minutes. Wash the cell pellet with 25ml of ice-cold, sterile 1X PBS and centrifuge at 300 × *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. Aliquot the preheated Dilution Buffer to a sterile tube and add 10.25µl of β-Mercaptoethanol (97.4%) 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 × *g* for 10 minutes at **room temperature** to clear the homogenate.
8. Proceed to Washing Streptavidin Paramagnetic Particles.



ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601



Promega

© 2000–2016 Promega Corporation. All Rights Reserved.

Printed in USA. Revised 8/16
Part #9FB047

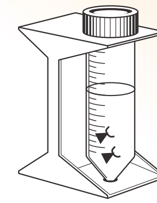
PolyATtract® System 1000

INSTRUCTIONS FOR USE OF PRODUCTS Z5400 AND Z5420.

Quick PROTOCOL

Washing Streptavidin Paramagnetic Particles (SA-PMPs)

1. Completely resuspend the SA-PMPs by gently rocking the bottle.
2. Transfer the 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 Stand toward the horizontal position until the particles are collected at the side of the tube. Carefully pour off the storage buffer.
3. Resuspend the 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.

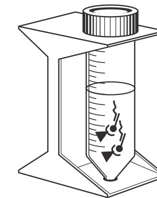


Wash SA-PMPs.

Capture and Washing

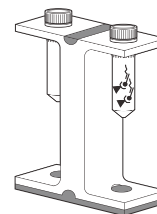
1. When centrifugation of the homogenate is complete, carefully transfer the supernatant to the tube containing the washed SA-PMPs in 0.5X SSC **away from the magnetic stand**. Mix by inversion.
2. Incubate the homogenate/SA-PMP mixture at room temperature for 2 minutes.
3. Capture the SA-PMPs by using 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.

Add supernatant to SA-PMPs.



Capture and concentrate particles.

Transfer to a 2ml mRNA User Tube.

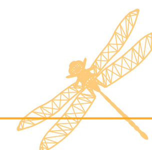


Capture and wash.

3047MA08_0A

ORDERING/TECHNICAL INFORMATION:

www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601



© 2000–2016 Promega Corporation. All Rights Reserved.

Printed in USA. Revised 8/16
Part #9FB047

PolyATtract® System 1000

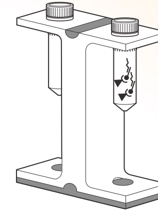
INSTRUCTIONS FOR USE OF PRODUCTS Z5400 AND Z5420.

Quick PROTOCOL

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 the 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 have been transferred, microcentrifuge the supernatant at 12,000 × *g* for 1 minute. Transfer the supernatant to a fresh tube and immediately place on ice.



Capture particles.

Elute mRNA.

Precipitate and concentrate mRNA.

3048MA08_0A

Precipitation and Concentration of mRNA

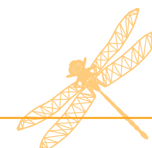
1. **For cDNA cloning:** Add 0.1 volume of 3M sodium acetate (pH 5.2) and 1.0 volume of isopropanol to the eluate and incubate at –20°C overnight.

   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 × *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 upon request from Promega or online at **www.promega.com***

ORDERING/TECHNICAL INFORMATION:
www.promega.com • Phone 608-274-4330 or 800-356-9526 • Fax 608-277-2601



Printed in USA. Revised 8/16
Part# 9FB047
© 2000–2016 Promega Corporation. All Rights Reserved.