# PolyATtract® mRNA Isolation Systems I and II

INSTRUCTIONS FOR USE OF PRODUCTS Z5200 AND Z5210.

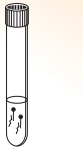
Quick PROTOCOL

## Large-Scale mRNA Isolation (1–5mg of total RNA)

*(For small-scale protocol, see reverse.)*

### Annealing of Probe

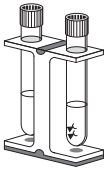
1. In a sterile, RNase-free 3ml tube, combine 1–5mg of total RNA and RNase-Free Water to a final volume of 2.43ml.
2. Heat at 65°C in a heating block for 10 minutes.
3. Add 10µl of the Biotinylated-Oligo(dT) Probe and 60µl of 20X SSC. Mix gently and incubate at room temperature until completely cooled.



Anneal probe.

### Washing Streptavidin Paramagnetic Particles (SA-PMPs)

1. Resuspend one tube of the SA-PMPs per isolation by gently flicking the bottom of the tube until they are completely dispersed. Capture the SA-PMPs by placing the tube in the Magnetic Stand.
2. Carefully remove the supernatant. (Do not centrifuge the particles.)
3. Wash the SA-PMPs three times with 0.5X SSC (1.5ml per wash). Following each wash, capture the SA-PMPs using the Magnetic Stand, and carefully remove the supernatant.
4. Resuspend the washed SA-PMPs in 0.5ml of 0.5X SSC.

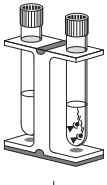


Wash SA-PMPs.

### Capture and Washing

1. Add the entire contents of the annealing reaction to the tube containing the washed SA-PMPs.
2. Incubate at room temperature for 10 minutes. Gently mix by inversion every 1–2 minutes.
3. Capture the SA-PMPs using the Magnetic Stand, and carefully remove the supernatant.
4. Wash the particles four times with 0.1X SSC (1.5ml per wash) by gently flicking the bottom of the tube until all of the particles are resuspended. After the final wash, remove as much of the supernatant as possible.

Add annealing reaction to SA-PMPs.



Capture and wash.

### Elution of mRNA

1. Resuspend the final SA-PMP pellet in 1.0ml of RNase-Free Water and gently resuspend the particles by flicking the tube.
2. Magnetically capture the SA-PMPs and transfer the eluted mRNA to a provided 2ml User Tube. (Save the particles.)

   **Note:** If any particles have been transferred, centrifuge at 12,000 × *g* for 1 minute at 4°C. Carefully transfer the RNA to a fresh RNase-free tube.

Elute mRNA.

*See additional protocol information in Technical Manual #TM021, available upon request from Promega or online at* ***www.promega.com***

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# PolyATtract® mRNA Isolation Systems III and IV

INSTRUCTIONS FOR USE OF PRODUCTS Z5300 AND Z5310.

Quick PROTOCOL

## Small-Scale mRNA Isolation (up to 1mg of total RNA)

*(For large-scale protocol, see reverse.)*

### Annealing of Probe

1. In a sterile, RNase-free 1.5ml tube, bring 0.1–1.0mg of total RNA to a final volume of 500µl in RNase-Free Water.
2. Heat at 65°C in a heating block for 10 minutes.
3. Add 3µl of the Biotinylated-Oligo(dT) Probe and 13µl of 20X SSC. Mix gently and incubate at room temperature until completely cooled.

### Washing Streptavidin Paramagnetic Particles (SA-PMPs)

1. Resuspend one tube of the SA-PMPs per isolation by gently flicking the bottom of the tube until they are completely dispersed. Capture the SA-PMPs by placing the tube in the Magnetic Stand.
2. Carefully remove the supernatant. (Do not centrifuge the particles.)
3. Wash the SA-PMPs three times with 0.5X SSC (300µl per wash). Following each wash, capture the SA-PMPs using the Magnetic Stand, and carefully remove the supernatant.
4. Resuspend the washed SA-PMPs in 100µl of 0.5X SSC.

### Capture and Washing

1. Add the entire contents of the annealing reaction to the tube containing the washed SA-PMPs.
2. Incubate at room temperature for 10 minutes. Gently mix by inversion every 1–2 minutes.
3. Capture the SA-PMPs using the Magnetic Stand, and carefully remove the supernatant.
4. Wash the particles four times with 0.1X SSC (300µl per wash) by gently flicking the bottom of the tube until all of the particles are resuspended. After the final wash, remove as much of the supernatant as possible.

### Elution of mRNA

1. Resuspend the final SA-PMP pellet in 100µl of RNase-Free Water and gently resuspend the particles by flicking the tube.
2. Magnetically capture the SA-PMPs and transfer the eluted mRNA to a sterile, RNase-free tube. (Save the particles.)
3. Repeat the elution step by resuspending the SA-PMP pellet in 150µl of RNase-Free Water. Repeat the capture step, pooling the eluate with the RNA eluted in Step 2 (250µl total volume).

   **Note:** If any particles have been transferred, centrifuge at 12,000 × *g* for 1 minute at 4°C. Carefully transfer the RNA to a fresh RNase-free tube.


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