POLYA+ mRNA PURIFICATION

Kit used: Dynabeads® mRNA DIRECT™ Kit (Invitrogen Cat. No. 61011)

Extra equipment needed: magnetic microcentrifuge tube rack

Material Guidelines

- Work RNase free
- Dissolve any precipitate in buffers before use by mixing at RT
- Bring Lysis/Binding Buffer and Washing Buffers to RT before use
- Elution Buffer should be stored on ice before use

Sample Preparation (Cultured Cells or Cell Suspension)

Note: protocol written for mRNA isolation on a micro scale (150,000 total cells or fewer). Consult the manufacturer's protocol for adjustments on scale.

1. Pellet the cells by a quick centrifugation (13,000 rpm for 30 sec)

   Note: for very small scale samples, i.e. 50 oocytes, transfer the cells to a drop of PBS before transferring to a microcentrifuge tube to more effectively wash in PBS. Store in -80°C until ready for use.

2. Wash the pellet: resuspend in PBS, repellet, store in -80°C or use immediately.

Dynabeads Preparation

1. Resuspend the Dynabeads thoroughly in the bottle before use.

2. Transfer 10 µL of beads to an RNase-free 1.5 mL microcentrifuge tube, place tube in magnet rack.

3. After 30 sec (or when suspension is clear), remove supernatant

4. Remove tube from magnet, wash by resuspending in ~50 µL Lysis/Binding Buffer.

mRNA Isolation

1. Add 300 µL of Lysis/Binding Buffer to each sample tube, mix by pipetting.

2. Remove the Lysis/Binding Buffer from the tube of Dynabeads: place tube on magnet rack and remove supernatant when clear.

3. Transfer the 300 µL of sample lysate into the tube with the beads and pipette to resuspend completely.

4. Incubate (continuous mixing, rotating or roller mixer) for 3-5 minutes at RT, allowing the mRNA to bind to the beads

5. Place tube on magnet rack for 2 minutes, remove supernatant.

6. Wash beads-mRNA complex 2X with 600 µL of Washing Buffer A at RT, using the magnet rack to separate the beads from the supernatant.

7. Wash beads-mRNA complex with 300 µL of Washing Buffer B at RT, place in magnet rack and remove supernatant.

8. For use in enzymatic downstream applications (e.g. RT-PCR): an extra wash in Washing Buffer B is recommended, followed by a final wash in the enzymatic buffer to be used.

9. For elution of mRNA, add 10 µL of Elution Buffer to the beads, mix by pipetting, and incubate between 65-80°C for 2 minutes. Immediately place tube on magnet, transfer eluted mRNA in the supernatant to a new RNase-free microcentrifuge tube on ice. Store at -80°C for later use.