

FastTrack® 2.0 Kit Checklist

Catalog nos. K1593-02, K1593-03

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Instructions*

- For lysates of tissue culture cells or tissues, start with Step 1.
- For total RNA, start with Step 6.

Isolating mRNA

- ☐ 1. Incubate the 15 ml lysate at 45°C, 15-60 minutes.
- ☐ 2. Centrifuge at 4,000 x g, 5 minutes, room temperature.
- ☐ 3. Transfer the supernatant to a new tube.
- ☐ 4. Add 950 µl 5 M NaCl stock solution and mix.
- ☐ 5. Shear DNA 3-4 times using an 18-21 gauge needle.
- ☐ 6. Add oligo(dT) cellulose to sample.
- ☐ 7. Incubate for 2 minutes, room temperature.
- ☐ 8. Rock the tube gently at room temperature, 15-60 minutes.
- ☐ 9. Centrifuge oligo(dT) at 3000 x g, 5 minutes, room temperature.
- ☐ 10. Remove the supernatant carefully from the oligo(dT) bed.
- ☐ 11. Proceed to the **Washing Procedure**.

* See manual for details on preparing lysates and total RNA.

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Washing Oligo (dT) cellulose

- ☐ 1. Resuspend oligo(dT) in **20 ml Binding Buffer**.
- ☐ 2. Centrifuge at 3000 x g, 5 minutes, room temperature.
- ☐ 3. Resuspend oligo(dT) in **10 ml Binding Buffer**.
- ☐ 4. Centrifuge at 3000 x g, 5 minutes, room temperature.
- ☐ 5. Resuspend oligo(dT) in **10 ml Low Salt Wash Buffer**.
- ☐ 6. Centrifuge at 3000 x g, 5 minutes, room temperature.
- ☐ ☐ ☐ 7. Repeat Steps 5-6 until the buffer is no longer cloudy.
- ☐ 8. Resuspend the oligo(dT) in **800 µl Low Salt Wash Buffer**.
- ☐ 9. Transfer the oligo(dT) to a spin-column.
- ☐ 10. Centrifuge at 5,000 x g, 10 seconds, room temperature.
- ☐ 11. Decant the liquid inside the microcentrifuge tube.
- ☐ 12. Repeat Steps 9-11 to transfer all of the cellulose to the spin-column.

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Washing Oligo (dT) cellulose, continued

- ☐ 13. Wash the oligo(dT) with ~500 μ l **Low Salt Wash Buffer**.
- ☐ 14. Centrifuge for 10 seconds, room temperature.
- ☐ ☐ ☐ 15. Repeat Steps 13-14 until the OD₂₆₀ of the “flow-through” is ~ 0.05.

Eluting and Precipitating the mRNA

- ☐ 1. Place the spin-column into a new microcentrifuge tube.
- ☐ 2. Resuspend the oligo(dT) in **200 μ l Elution Buffer**.
- ☐ 3. Centrifuge for 30 seconds, room temperature. **Do not decant.**
- ☐ 4. Resuspend the oligo(dT) in a second **200 μ l aliquot Elution Buffer**.
- ☐ 5. Centrifuge for 30 seconds, room temperature.
- ☐ 6. **Save** the 400 μ l eluent in the microcentrifuge tube.
- ☐ 7. Add 60 μ l of 2 M sodium acetate (supplied) and 1 ml of 100% ethanol.

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Eluting and Precipitating the mRNA, continued

- ☐ 8. Freeze on dry ice until solid.
- ☐ 9. Thaw and centrifuge at 16,000 x g, 15 minutes, +4°C.
- ☐ 10. Remove all the ethanol.
- ☐ 11. Resuspend the RNA pellet in **20-50 µl Elution Buffer.**
- ☐ 12. Determine the concentration of the mRNA.
- ☐ 13. Store mRNA at -70°C or use immediately.

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