

Purification of polyA⁺RNA from total RNA.

Sample preparation:

1. Thaw 50 µg total RNA quickly.
2. Add DEPC-treated H₂O to 50 µL and 50 µL 2× binding buffer
3. Heat the RNA 10 min. 65 °C and quench on ice 10 min.

Oligo-T pre-coating of streptavidin-coated magnetic particles

4. Pipette 60 µL of streptavidin-coated magnetic particles into a tube for each sample
5. Use the magnetic separator and remove the supernatant
6. Optional: Add 100 µL 0.5 µg/µL yeast tRNA diluted in TE buffer.
7. Optional: Pre-block for 5 min at room temperature (RT)
8. Add 200 pmol biotinylated oligo-T LNA/DNA capture probe (product no. 300100-B) diluted in 100 µL TE buffer to the streptavidin-coated magnetic particles
9. Incubate 5 – 10 min at 37 °C (shaking gentle or rotation).
10. Wash the particles in 100 µL TE buffer and twice in 1× binding buffer.
11. The particles are equilibrated in the adequate buffer used for binding the polyA⁺RNA.

Purification protocol

12. Transfer the sample to the pre-coated magnetic particles
13. Incubate 5 min 37 °C (shaking, 400 rpm)
14. Wash the particles in low salt washing buffer three times.
15. Finally, dilute in 20 – 50 µL DEPC-H₂O.
16. Incubate at 65 °C 10 min for releasing the polyA⁺RNA from the particles and quench on ice for 5 min.
17. Spin briefly (13.2 rpm 60 sec) and transfer the supernatant to a clean siliconized eppendorf tube. To avoid any magnetic particles to disturb the absorbance measurement.

Quantification (Optional)

18. Apply 1 µL of the polyA⁺-selected RNA on a native 1-% agarose gel in 1x TAE buffer containing 1:10000 Gelstar.
19. Run the agarose gel for ca. 60 min 7 V/cm
20. Scan the agarose gel on the Amersham Pharmacia Biotech Typhoon 9200 Imager and quantify the quality of the polyA⁺-selected RNA.

Buffers:

2× binding buffer: 40 mM Tris-HCl (pH 7.5), 0.2 M NaCl, 2 mM EDTA (pH 7.5) 0.2%(w/v) N-lauryl sarcosinate

TE buffer: 10 mM Tris-HCl, 1 mM EDTA, pH 7.5

Binding/washing buffer low salt (20 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 1 mM EDTA (pH 7.5) 0.1%(w/v) N-lauryl sarcosinate)