TU-BIOTINYLATED RNA PURIFICATION

1. µMacs Streptavidin Kit (Miltenyi, Order No. 130-074-101) only use the beads, not the supplied buffers

2. Magnetic Stand from Miltenyi (one stand holds 4 columns) => has to fit the small columns of the µMacs Streptavidin Kit

3. Washing Buffer (50 ml): 100 mM Tris pH 7.5, 10 mM EDTA, 1 M NaCl, 0.1% Tween 20.

** Heat Washing Buffer to 65°C (DO THIS BEFORE STARTING).

4. Elution Buffer (always prepare fresh): 5% b-ME in RNAse-free water.

PROTOCOL:
1. Heat biotinylated RNA samples to 65°C for 10 min and immediately place on ice for 5 min. Add up to 100 µg (max. 100 µl) of biotinylated RNA to 100µl of streptavidin beads. Incubate with rotation for 15 min.

Small differences in sample volume added to the columns do not matter. In case RNA input volumes vary by >2-fold simply add the required volume of 1x TE to the beads to equalize conditions (i.e. if one sample is 100 µl of RNA and another sample is only 40 µl of RNA, add 60 µl of TE to the mixture of beads and RNA for this second sample: both samples will then be incubated in 200 µl volume of RNA + beads)

2. Place µMacs columns into magnetic stand. Do not process more than 12 samples at a time (6-8 samples are optimal).

3. Add 0.9 ml of washing buffer to columns, let flow through. (this "pre-run" equilibrates the column, do this just before adding the beads + RNA mixture).

4. Apply beads + RNA mixture to the columns. Optional: collect the flow-through into 1.5 ml tube and save on ice (can use this to precipitate RNA if desired, see below).

5. Wash 3x with 0.9 ml 65°C washing buffer.

6. Wash 3x with 0.9 ml room temperature washing buffer. Optional: collect last wash for RNA precipitation.

7. Pipet 700 µl Buffer RLT (RNeasy MinElute Cleanup Kit) into new 2 ml tubes. Elute RNA directly into Buffer RLT by placing the tubes underneath the columns and adding 100 µl Elution Buffer (5% b-ME) to the columns. Perform a second elution round 3 min later.

8. Continue with the RNease MinElute Cleanup Protocol following the manufacturer's instructions shown below.

9. Add 500 µl 100% ethanol to the diluted RNA and mix thoroughly by pipetting. Do not centrifuge.

10. Apply 700 µl of the sample to an RNAeasy MinElute Spin Column in a 2 ml collection tube. Close the tube gently and centrifuge for 15 s at >8000 g. Discard the flow-through. Apply the remaining 700 µl and repeat the centrifugation. Discard the flow-through.

11. Transfer the spin column into a new 2 ml collection tube. Pipet 500 µl Buffer RPE onto the spin column. Close the tube gently and centrifuge for 15 s at >8000 g to wash the column. Discard the flow-through.

12. Add 500 µl of 80% ethanol to the spin column. Close the tube gently and centrifuge for 2 min at >8000 g to dry the silica-gel membrane. Discard the flow-through and collection tube.

13. Transfer the spin column into a new 2 ml collection tube. Open the cap of the spin column and centrifuge at full speed for 5 min. Discard the flow-through and collection tube.

14. To elute, transfer the spin column to a new 1.5 ml collection tube. Pipet 15 µl RNase free-water directly onto the center of the silica-gel membrane. Close the tube gently and centrifuge for 1 min at maximum speed to elute.

15. Store RNA at -80 °C until ready for analysis.
To precipitate RNA from the "unbound" and "last wash" fractions:
1. Add equal volume of isopropanol. For last wash, split sample into two 450 µl samples (two different 1.5 ml tubes), add 450 µl isopropanol to each.
2. For last wash sample, add 1 ml linear acrylamide (carrier to aid in precipitation of RNA).
3. Incubate samples at RT for 5 minutes then centrifuge in a microcentrifuge at max speed for 20 min, at 4°C
4. Resuspend unbound sample RNA in volume equal to the starting volume of biotinylated RNA at the beginning of the purification.
5. Resuspend last wash sample RNA in 10 µl: first add 10 µl to one of the two tubes, pipette up and down and mix at bottom of tube to collect any RNA, then transfer that 10 µl to the second tube, repeat mixing.
6. Take spec. readings of the unbound and last wash samples. Calculate amount of RNA in each.