Size-limited purification of RNA

Isolation of total RNA by the NucleoSpin miRNA-kit by MACHEREY-NAGEL:

The RNA is purified by size digestion so that the purified RNA has a length of < 200 nucleotides. This has the advantage that the proportion of tRNA contained therein is considerably increased compared to the total RNA.

1. Cell lysis:
    a. Elution of the biomass pellet in 300 µL of ML solution
    b. Rest for 5 min at room temperature
2. Homogenization of the lysate:
    a. Load sample to the Filter Column with the violet ring
    b. Centrifuge it for 1 min at 11.000g
    c. Discard the Filter Column
3. Adjust binding conditions for large RNA and DNA:
    a. Add 150 µL of 96-100% ethanol to the sample
    b. Vortex it for 5 s
    c. Rest for 5 min at room temperature
4. Bind large RNA and DNA:
    a. Load sample to the Filter Column with the blue ring
    b. Centrifuge it for 1 min at 11.000g
    c. Discard the Filter Column
5. Precipitate protein:
    a. Add 300 µL of the MP-solution to the sample
    b. Vortex it for 5 s
    c. Centrifuge it for 3 min at 11.000g
6. Remove residual debris:
    a. Load supernatant to the Filter Column with the white ring
    b. Centrifuge it for 1 min at 11.000g
    c. Discard the Filter Column
7. Adjust binding conditions for small RNA:
    a. Add 800 µL MX-solution to the sample
    b. Vortex it for 5 s
8. Preparation of the blue Filter Column:
    a. Add 300 µL ML-solution and 150 µL 96-100% ethanol to the column
    b. Centrifuge it for 1 min at 11.000g
    c. Add 350 µL MDB-solution to the column
    d. Centrifuge it for 1 min at 11.000g
9. Bind small RNA:
    a. Load 600 µL of the sample to the blue Filter Column
    b. Centrifuge it for 30 s at 11.000g
    c. Repeat that for two times

10. Wash and dry silica membrane:
    a. Add 600 µL of MW1 to the blue Filter Column
    b. Centrifuge it for 30 s at 11.000g
    c. Add 700 µL of MW2 to the blue Filter Column
    d. Centrifuge it for 30 s at 11.000g
    e. Add 700 µL of MW2 to the blue Filter Column
    f. Centrifuge it for 30 s at 11.000g
    g. Add 250 µL of MW2 to the blue Filter Column
    h. Centrifuge it for 2 min at 11.000g
11. Elute RNA:
    a. Add 50 µL of RNase-free H2O to the blue Filter Column
    b. Rest for 1 min at room temperature
    c. Centrifuge it for 1 min at 11.000g Centrifuge it for 2 min at 11.000g