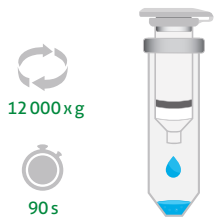
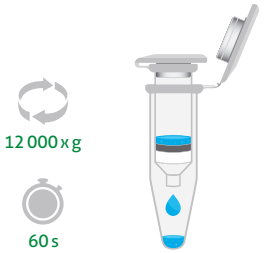
STEP 8



Centrifuge for 90 s at ≥ 12 000 x g (preferably at 15 000 x g). Discard the collection tube and the filtrate and carefully transfer the purification minicolumn to a sterile RNase-free 1.5 ml Eppendorf tube.

⚠ RW2 Buffer contains alcohol, which may interfere with some enzymatic reactions and decrease the elution efficiency. It is therefore crucial to remove the alcohol completely from the minicolumn before elution.

STEP 9



Add **50-100 µl** elution buffer **REB**. Centrifuge for 60 s at ≥ 12 000 x g to elute purified RNA.

The isolated RNA is ready for use in downstream applications.

⚠ Other buffer volumes in the 30-50 µl range may be used. For instructions, see to section VIII. Recommendations and important notes.

EXTRACTME® TOTAL RNA KIT

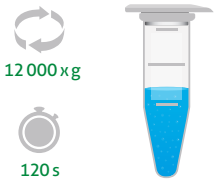
Cat. No. EM09.1, EM11.1
Version: 1.2017

ISOLATION PROTOCOL

STEP 1

Place a fragmented biological material in a 2 ml tube. Add **600 µl RLys Buffer** and **20 µl AF Reagent** and vortex for 60 s.

STEP 2



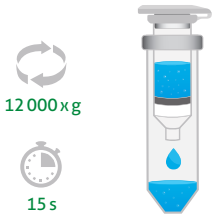
Centrifuge for 120 s at ≥ 12 000 x g (preferably at 15 000 x g).

STEP 3

Transfer the supernatant into an **RNase-free 1.5-2 ml** Eppendorf tube and add **600 µl 70% ethanol** to the transferred supernatant. Mix well by pipetting or vortexing.

⚠ For homogenization with the use of bead-beating tubes: carefully pipet an appropriate volume of the supernatant by placing a 200 µl pipette tip (N.B.: a 1 ml tip may be clogged by the beads) into the filling. Tissue remains should either lie on one side of the tube or at the bottom.

STEP 4



Transfer up to **700 µl of the obtained mixture** into an **RNA Purification Column** placed in a collection tube. Centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the column together with the collection tube.

Transfer the **remaining mixture** into the same purification minicolumn and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and place the minicolumn in a new collection tube.

STEP 5 / OPTIONAL (DNase treatment)

a. Prewash the minicolumn with **500 µl RW2 Buffer** and centrifuge for 60 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

b. For each isolation mix **90 µl 10x DNase I Reaction Buffer** and **10 µl reconstituted DNase I**. Mix by inverting the tube.

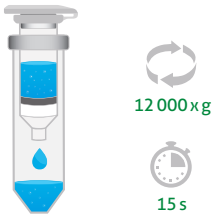
c. Apply **95 µl mixture** onto the center of the RNA Purification Column. Incubate for 5 minutes at room temperature.

d. Add **600 µl RW1 Buffer** and centrifuge for 15 s at 12 000 x g. Discard the filtrate and reuse the collection tube. Proceed to **step 7**.

STEP 6 / omit after DNase treatment

Add **700 µl RW1 Buffer** and centrifuge for 15 s at ≥ 12 000 x g.

Discard the filtrate and reuse the collection tube.



STEP 7

Add **500 µl RW2 Buffer** and centrifuge for 15 s at ≥ 12 000 x g. Discard the filtrate and reuse the collection tube.

Repeat step 7.