RNA Isolation Protocol

1. **Place** the fragmented biological material in a 2mL tube.
   Add 600µL RLys Buffer and vortex for 60 seconds

2. **Centrifuge** for 120 seconds at ≥12000 x g (preferably at 15000 x g).

3. **Transfer** supernatant into an **RNase-free** 1.5 or 2.0mL reaction tube and add **600µL 70% ethanol** to the transferred supernatant.
   Mix by pipetting or vortexing.
   NOTE: For homogenization using bead-beating tubes: carefully pipet the appropriate volume of the supernatant by placing a 200µL pipette tip (N.B.: a 1mL 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.

4. **Transfer** up to **700µL of the obtained mixture** into an **RNA Purification Column** placed in a collection tube. Centrifuge for 15 seconds at ≥12000 x g.
   Discard flow-through and re-use the column, together with the collection tube.

5. **Transfer** the **remaining mixture** into the same purification column and centrifuge for 15 seconds at ≥12000 x g.
   Discard flow-through and place the mini column in a new 2mL collection tube.

---

6. Optional - On-column DNase treatment

a. Prewash mini column with 500µL **RW2 Buffer** and centrifuge for 60 sec. at ≥12000 x g.
   Discard flow-through and re-use the collection tube.
b. For each isolation mix 90µL **DNase I Reaction Buffer** and 10µL reconstituted **DNase I** (**not included in this kit**). Mix by inverting the tube.
c. Apply 95µL of the above mixture onto the centre of the RNA Purification Column.
   Incubate for 5 minutes at room temperature.
d. Add **600µL RW1 Buffer** and centrifuge for 15 sec. at ≥12000 x g.
   Discard flow-through and re-use the collection tube.
   Proceed with step 8.

---

7. **O**mit this step in the case of DNase I treatment / step 6)
Add 700µL RW1 Buffer and centrifuge for 15 sec. at ≥12000 x g.
Discard flow-through and reuse the collection tube.
NOTE: Do proceed with step 8 directly without step 7 if the optional DNase I treatment was done, as described in step 6.

8. **Add 500µL RW2 Buffer** and centrifuge for 15 sec. at ≥12000 x g.
   Repeat this step.

9. Centrifuge for 90 sec. at ≥12000 x g (preferably at 15000 x g). Discard the collection tube and the flow-through and carefully transfer the Purification Column to a RNase-free 1.5mL reaction tube.
   NOTE: 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 mini column before elution.

10. **Add 50-100µL elution buffer REB** precisely onto the centre of the purification column membrane. Centrifuge for 60 sec. at ≥12000 x g to elute purified RNA.
    The isolated RNA is ready for use in downstream applications or for storage at -80°C.
    NOTE: Other buffer volumes in the range of 30-50µL may be used. For instructions, see page 8 (RNA elution) (Recommendations and important notes).

RNA Clean-up Protocol (for already isolated RNA)

1. Adjust RNA sample volume to **100µL** by adding RNase-free water to a 1.5 or 2.0mL RNase-free reaction tube.
2. Add **300µL RLys Buffer**.
3. Add **300µL 96-100% ethanol**. Mix well by pipetting or vortexing.
4. Transfer the mixture into a **RNA Purification Column** placed in a collection tube.
   Centrifuge for 15 sec. at ≥12000 x g.
   Discard the flow-through and re-use the collection tube.

   Proceed to **step 7** of the Isolation Protocol (page 7)