RNA isolation using TRI Reagent and ReliaPrep kit

- Add approx. 1ml of ceramic beads (1.4mm) to the tissue in 1ml of TRI Reagent on ice.
- Disrupt the tissue in homogenizer (Precellys Evolution – 6,500 RPM for 50 seconds / Bead Ruptor – speed 5 for 50 seconds)
- If pieces of tissue are still visible, incubate the tube on ice for at least 1 minute and repeat the disruption step.
- Add 100 µl BCP solution to the tube, mix well, and incubate 5-15 minutes at RT.
- Centrifuge at 12,000 x g for 10-15 minutes at 4°C then transfer the aqueous phase to the new tube.
- Add 70% EtOH to the aqueous phase in volume ratio 1:1 and mix well.
- Transfer 500 µl of resulting solution to a ReliaPrep Minicolumn and centrifuge at 12,000-14,000 x g for 30 seconds at RT.
- Discard the liquid in the collection tube.
- Repeat the step with solution transfer until nothing is left.
- Discard the liquid in the collection tube.

Continue with the manufacturer's protocol ReliaPrep RNA Cell Miniprep System from step 8 / ReliaPrep RNA Tissue Miniprep System from step 6

- Add 500 µl of RNA Wash Solution to the column and centrifuge at 12,000-14,000 x g for 30 seconds at RT.
- Discard the liquid in the collection tube.
- In a sterile tube, prepare DNase I incubation mix for all samples, volumes per sample are:
    - 24 µl of Yellow Core Buffer
    - 3 µl of 0.09M $MnCl_2$
    - 3 µl of DNase I enzyme (do not vortex)
- Mix the DNase I incubation mix by pipetting, do not vortex.
- Apply 30 µl of freshly prepared DNase I incubation mix directly to the membrane inside the column. Make sure the mix is fully covering the membrane and incubate for 15 minutes.
- After incubation, add 200 µl of Column Wash Solution to the column.
- Centrifuge the column at 12,000-14,000 x g for 15 seconds (no need to empty the collection tube after).
- Add 500 µl of RNA Wash Solution to the column and centrifuge at 12,000-14,000 x g for 30 seconds.
- Discard the collection tube and place the column into a new one.
- Add 300 µl RNA Wash Solution to the column and centrifuge at high speed for 2 minutes.
- Discard the collection tube and place the column into capped 1.5 ml elution tube.

- Add Nuclease-Free Water directly to the membrane according to the table below. Make sure the Nuclease-Free Water is fully covering the membrane and centrifuge for 12,000-14,000 x g for 1 minute.

Input range	Nuclease-Free Water
$1x10^2$ to $5x10^5$ of cells	15 µl
> $5x10^5$ to $2x10^6$ of cells	30 µl
> $2x10^6$ to $5x10^6$ of cells	50 µl
5mg of tissue or less	15 µl
> 5mg of tissue	30 µl

- Cap the elution tube and put on ice / store at -80°C.