**Protocol for DNasing with the RNAqueous-Micro Kit**

**Before Beginning**

* Put the 10X DNase I Buffer and the DNase Inactivation Reagent (bead slurry) on ice to thaw
* Transfer your eluted RNA into sterile, clearly labeled 0.5 ml tubes
* Heat either a heat block or a water bath to 37 °C

1. **If working with more than ~4 samples, prepare a master mix**
   * For each sample, you will need 1/10 of your RNA volume of 10X DNase I Buffer. For example, if you are working with RNA eluted in 20 µl, you will need 2 µl of 10X buffer for each sample
   * For each sample, you will also need 1 µl of DNase I (pulled from freezer when ready)
     1. Flick the enzyme to mix it well
     2. Pipette ***SLOWLY***
   * Always prepare a master mix that has slightly more volume than you actually need to account for evaporation and loss to the exterior of the pipette tips
   * When you add from the master mix to each sample, back-pipette gently to the first stop several times to ensure all of the mix was ejected; then, eject to the second stop
2. **If ≤ 4 samples**
   * When you add the 10X DNase I Buffer, eject into the sample liquid
   * When you add the enzyme, back-pipette slowly to the first stop several times to ensure all of the enzyme was ejected; then, eject to the second stop
3. **Incubate the reaction at 37 °C for 30 minutes**
   * This can either be done in the water bath or the heat block, even without adapters
4. **Add the DNase Inactivation Reagent** 
   * Vortex the DNase Inactivation Reagent (bead slurry) to ensure an even suspension of the microscopic inactivation beads
   * Add 2 µl OR 1/10 RNA volume to each sample, whichever is greater (2 µl for 20 µl RNA eluate)
   * Eject into the sample; you will see the inactivation reagent sink to the bottom of the tube
   * After adding to each tube, gently flick to mix, then tap on the bench to remove from the walls of the tube (rather than spinning)
   * Wait 1 minute at RT
   * Flick again, tap down again, wait 1 minute at RT again
5. **Spin down the beads and transfer the DNased RNA**
   * Centrifuge for 2 minutes at max speed to pellet the beads in the bottom of the tube
   * Carefully pipette out the DNased RNA on top of the beads; try to recover as much as possible
   * For a 20 µl RNA volume, the final volume in the tube at this stage should be 25 µl; try to recover ~21-22 µl
   * Transfer to a final, labeled tube that includes “DNased” somewhere