

Supplemental protocol: Measure RNA Concentration and Purity

1. Resuspend RNA pellet in 50-100 μ l TE or H₂O (or sufficient volume)
2. Make sure the RNA is in solution: mix by pipetting or vortexing repeatedly.
3. Dilute sample 1:40 (2 μ l sample in 78 μ l H₂O) and 1:100 (2 μ l sample in 98 μ l H₂O).
4. Soak cuvettes in 1:1 solution of Methanol:12N HCL Acid for 20 minutes, wash with DEPC treated water, dry well and visually inspect for cleanliness.
5. Load cuvettes into their holder (in lab) and place in Spec. Set the Spec to read Absorbance at 260, 280, 320nm.
6. Fill each cuvette with 100 μ l DEPC treated water and remember to blank the machine. Then record the A₂₆₀ for each of the 5 water samples.
7. Remove each water sample (except the first one) and add diluted RNA. Record the Absorbance, making sure you know what sample corresponds to which cuvette.

Make sure the Absorbance of the first cuvette does not “drift” too much. If you are analyzing multiple samples (>10) and the Absorbance begins to “drift”, you will need to repeat step 6 and average the two Absorbance values.

8. The A₂₆₀ of each sample should be between 0.1 and 1, this is within the linear range of the instrument, you can perform two dilutions to make sure this is the case. A₃₂₀ will give an estimate of contamination, the lower the value the better. A₃₂₀ of each sample should be less than 0.05.
9. Clean out the cuvettes between samples by adding 100 μ l DEPC treated H₂O.
10. Determine the RNA concentration of each sample. Make sure to subtract the A₂₆₀ of water for each cuvette from each sample. Even if the A₂₆₀ value is negative, still subtract.

If A₂₆₀ = 1, then the RNA concentration is approximately 40 μ g RNA/ml. Following are the calculations used to arrive at this concentration:

$$A_{260} * 40 = \mu\text{g RNA/ml}$$

$$A_{260} * 40 / 1000 = \mu\text{g RNA}/\mu\text{l}$$

For a solution diluted 100-fold, we must multiply the left side by 100:

$$A_{260} * 40 * 100 / 1000 = \mu\text{g RNA}/\mu\text{l}$$

For a solution diluted 40-fold, we must multiply the left side by 40:

$$A_{260} * 40 * 40 / 1000 = \mu\text{g RNA}/\mu\text{l}$$

Supplemental Protocol: RNase Free glassware and reagents.

Sterile, disposable plasticware is generally free of RNase activity.

Glassware should be treated by baking at 250 C for >4 hours. In addition, treatment with a 0.1% solution of diethylpyrocarbonate (DEPC), an inhibitor of RNase is sometimes used prior to autoclaving and baking glassware.

DEPC treated H₂O is also used for preparing reagents. Add 1ml DEPC per L of water, and let sit for 12 hours, then autoclave for 15-20 min. Use this treated water for preparing all solutions. Note that DEPC cannot be used to treat Tris buffered solutions as it will degrade into ETOH and carbon dioxide.

Hands are a major source of RNase contamination. Use gloves at all stages of preparation.

Segregate RNase free glassware and reagents in the lab.

Many companies offer RNase inhibitors that can be incorporated into protocols. Browse catalogs for more information.