

## Total tRNA Sample preparation for LC-MS/MS by Limbach Lab

Any standard total tRNA purification approach is, generally speaking, compatible with downstream mass spectrometry. The key is to remove any non-MS friendly reagents by precipitation using 7.5-M ammonium acetate. Do not use sodium, potassium or Tris-based salts for tRNA precipitation.

1. To 1 volume of RNA (say 100  $\mu$ l), add 1/3 volume of 7.5M ammonium acetate (33.3 $\mu$ l), 2.5 volumes of absolute ethanol (250 $\mu$ l), invert a few times and incubate at -20 °C for at least 3 hr or -80 °C for 1 hr.
2. Centrifuge the suspension at 12000 x g for 15 min. Decant the supernatant, invert the tube on Kim wipe to remove residual liquid and dry the pellet at 65 °C for 10 min before dissolving in sterile water.
3. Repeat the procedure (steps 1 and 2) for one or two more times depending on the initial total tRNA preparation procedure. Procedures using fractionation columns tend to have significant salt levels. PAGE gel purification of tRNAs will usually have lesser amounts.
4. **Important:** Before the final precipitation, obtain a concentration reading of the sample amount being shipped. Take measurements at 260 nm and 280 nm to gauge tRNA purity and amount. Samples are best shipped after centrifugation and drying (see Step 2). Provide the sample amount (if reporting concentration, be sure to include original sample volume during measurement; otherwise calculate mass-based amount of total tRNA in each microcentrifuge tube). Do not send samples that do not contain the requested amount of total tRNA, which depends on the analysis required and will be communicated to you by the lab before sample preparation begins.

Samples should be shipped overnight with the lab notified of shipping information so that someone is present to accept the shipment. Do not ship on Friday or before holidays as there is no guarantee anyone will be present at the University to accept the shipment.

Once samples are received, they will be reconstituted in sterile water, sample concentration/amounts will be verified, and if sufficient sample is present, analysis by mass spectrometry can proceed.