Acetone/Ethanol Preparation of Bacteria for RNA samples

This protocol is used to store bacterial cultures and to immediately arrest RNA degradation. Samples can be stored for a long period of time at -20C before RNA is extracted.

Solutions:
Acetone/EtOH - 1:1 vol/vol ratio
PBS (as per Maniatis)
Isopropanol - RNAse free
70% Ethanol - RNAse free
95% Ethanol - RNAse free
DEPC-treated water - RNAse free
TE Buffer (as per Maniatis) - RNAse free

Equipment:
25 mL Corning glass tubes
-Tubes should be silicon-coated and allowed to bake at 180C O/N, to degrade all RNAses.

Caps for Corning glass tubes
-These caps should be sprayed with RNAZap, and allowed to sit in this solution for 5-10 minutes. The caps are then washed with a generous portion of DEPC-treated water. This water can then be dried off by either laying the cap face down on a fresh Kimwipe or pouring a small portion of RNAse free EtOH into the cap and then allowing to air dry face down on a Kimwipe.

Protocol:
l) Grow organisms to desired optical density (OD), immediately take 1 volume of that culture and place into 1 volume of ice cold Acetone/EtOH (ie: 10 mL of culture into 10 mL of the Acetone/EtOH mixture).

Invert tube 10 times, make sure tube is labeled and place at -20C.
-Samples can be placed into Sarstedt tubes (50 mL conicals), which according to the manufacturer do not have any adverse effects when acetone and/or ethanol are stored in them for longer periods of time.

2) When an RNA preparation of the sample is desired, take the tube and spin at 3,000g for 5 minutes to lightly pellet the cells. After this pelleting, pour off the Acetone/EtOH and resuspend in 5 mL of cold PBS. Mix cells to wash and then spin again at full speed for 5 minutes.

3) Once again, pour off spent PBS wash, resuspend in 1 mL of fresh PBS move to a 25 mL Corning glass tube (silicon-coated)and add 5 mL of RNAzol. Mix by vortexing, then add 0.1 volume of chloroform(600 microliters), mix again and allow to sit on ice for 5 mdnutes to get a tight interface and then spin at 12,000g (ref) for 15 minutes at 4C.

4) Take aqueous phase (top portion) and place into a clean 25 mL Corning tube. Add an equal volume of isopropanol. Invert a few times and then spin at 12,000g for 15 minutes at 4C. You should see a white pellet at the bottom of the tube at this point. Wash once with RNAse-free 70% EtOH, spin again at 12,000g for 15 minutes at 4C.

5) Pour off the 70% EtOH, invert tube to let residual EtOH run out of tube. Be VERY careful at this point as RNA pellet may be loose and may start to slide down the side of the tube. Allow to air dry. At this point, the RNA should look like a small droplet of water on the side of the tube. This is your RNA. Resuspend in 100 microliters of RNAse-free TE buffer and freeze at -20C (if to be used immediately) or -80C (for long term storage).