Isolation of Bacteria Using the Total RNA Kit I (R6834)

1. Grow Bacteria in LB media to log phase. (Do not use an overnight culture.)

2. Harvest no more than 3 ml culture (<1 x 10^9 bacteria) by centrifugation at 4,000-5000 x g for 5- min at 4°C.

3. Resuspend the Bacterial Pellet with the appropriate amount of TE Buffer Containing Lysozyme (1 mg/mL for Gram Negative, 4 mg/mL for Gram Positive). Incubate at room temperature for 4 minutes.

3. Discard medium and resuspend cells in the appropiate amount of TRK Lysis Buffer according the chart below. Mix by pipetting up and down 5-10 times.

Number of Cells	Amt of TE Buffer(containing Lysozyme)	Amt of EtOH (96-100%)	Amount of TRK Lysis Buffer
<5 x 10^8	100 µl	250 µl	350 µl
5 x 10^8 – 1 x 10^9	200 µl	500 µl	700 µl

4. Add the appropriate amount of EtOH to the sample according to the chart above. Mix by Vortexing.

5. Follow the Total RNA Kit I protocol from Step 6 of the Animal Cells Protocol