Isolation of crude mitochondria from cell lines

1. Prepare fresh Isolation Buffer and chill on ice:
   0.3 M mannitol (Sigma) 
   0.1% BSA (Sigma) 
   0.2 mM EDTA 
   10 mM HEPES 
   adjust pH 7.4 with KOH 
   1X protease inhibitor cocktail (1/25 dilution Complete, Roche)

2. Trysinize cells ($10^7$) wash with cold PBS.

3. Resuspend in cold Isolation Buffer (use 5 times pellet volume).

4. Homogenize on ice with a 2-ml glass homoginizer (Dounce: loose x 5 times then tight x 5 times).

5. Centrifuge 1000g at 4°C for 10 min. Collect supernatant. Discard the pellet (whole cells and nuclei).

6. Spin 14,000g for 15 min at 4°C, and the resulting supernatant is saved as the cytosolic fraction.

7. The resulting pellet represents the mitochondrial fraction, and is washed twice with cold Isolation Buffer.
