Cytochrome c Assay

(Waterhouse, NJ, Steel, R, Kluck, R, and Trapani, JA. Assaying cytochrome C translocation during apoptosis. Methods Mol Biol. 2004;284:307-13)

Permeabilization Buffer:

80 mM KCl in PBS
+ digitonin (made and added fresh)

For BU-11, add 20 μl PB + 0.625 μl/ml of 100 μg/ml digitonin in DMSO per $1*10^6$ cells. For other cell lines, titrate cell number and buffer volume so that 95% of cells are trypan blue positive.

Total Cell Lysis Buffer:

50 mM Tris-HCl, pH 7.4
150 mM NaCl
2 mM EGTA
2 mM EDTA
0.2% Triton X-100
0.3% NP-40
Protease inhibitor cocktail

1. Resuspend cells in PB and incubate on ice for 5 min.
2. Check an aliquot for permeabilization by Trypan blue.
3. Centrifuge lysates (800 g for 5 min at 4°C).
4. Transfer super to new tube and determine protein concentration.
5. Resuspend the pellet in 100 ul TCLB per 3*106 cells and rock at 4°C for 10 min.
6. Centrifuge lysates (max g for 10 min at 4°C).
7. Transfer super to new tube and determine protein concentration.