Procedure for apoptosis/necrosis cell analysis
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Vybrant Apoptosis Assay Kit #3
FIC annexin V/propidium iodide
Invitrogen # V13242

Positive controls:

1. Apoptosis: 0.1% sodium iodide in cell culture media. Applied 24 hours before experiment. Cells maintained incubator.
2. Necrosis: Cells exposed to ice for approximately 30 minutes before staining.

Negative controls:

1. Cells cultured on tissue culture polystyrene without any changes.

Note: For 200 microliters of final volume for 5 wells. Good for 12 and 24-well plates. Okay for 6-well plates.

Steps:

1. Make 3 mL of 1x annexin-binding buffer
    a. 600 microliter of 5x buffer (Component C)
    b. 2400 microliter of nanopure water, sterile
2. Make 25 microliters of 100 micrograms/mL PI solution
    a. 2.5 microliters of PI solution (Component B)
    b. 22.5 microliters of 1x annexin-binding buffer (from #1)
3. Rinse cells in cold PBS. Aspirate off.
4. Replace media with 200 microliters of annexin-binding buffer per well
5. Add 10 microliters of Annexin V conjugate (Component A) to each well
6. Add 4 microliters of 100 microgram/mL PI solution (from #2) to each well
7. Incubate at room temperature for 15 minutes.
8. Wash cells with 1x annexin-binding buffer. Aspirate
9. Add 500 microliters of PBS to each well, for 6-well. Less for 12 or 24 wells.

- Live cells should show weak FITC (green) staining due to staining of cellular membrane.
- Apoptotic cells should show a significantly higher degree of surface labeling. (FITC)
- Dead cells will show both membrane stains. The PI (red) stain should be a nuclear stain.