THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center
Making Cancer History®

APOPTOSIS STAINING GUIDELINES

PROCEDURE

1.0 Preparation

1.1 Prepare cells into a single cell suspension. Ensure that the final wash is done with PBS, and cells are resuspended in 1X binding buffer. Cells should be concentrated to approximately 1-5 million cells/ml.

Note: Calcium is required for Annexin V to bind, so do not use a buffer with EDTA or any other chelator.

1.2 Controls for this experiment should include an unstained tube, Annexin V only, PI only, and a tube treated with something to induce apoptosis as a positive control.

2.0 Staining

2.1 Distribute approximately 100,000 cells per tube.

2.2 Stain with Annexin V and PI according to manufacturer's recommendations, and vortex.

Note: Kit binding buffers are sometimes 10X. Always prepare the correct 1X dilution before adding.

2.3 Incubate for 15-20 minutes at room temperature, protected from light. Add up to 400 µl of binding buffer.

2.4 Analyze cells on the cytometer within 1 hour of staining.

RELATED PROCEDURES

This handout is related to ACSF SOP EX004. It is a handout only.