



## FITC Annexin

### Materials Provided:

- FITC Annexin V conjugate

### Materials Not Included:

- Flow Cytometry Staining Buffer (Stain Buffer) (1X PBS with 2% FBS, 0.09% Na-Azide)
- Annexin V Binding Buffer (1X) **CAT# TNB-5000-L050**
- 7-AAD Solution **CAT# 13-6993-T200**

### Procedure:

1. Wash cells with 1-2 mL Stain Buffer. Centrifuge at 300-400 x *g* for 5 minutes at room temperature and discard supernatant. Briefly vortex to dissociate the cell pellet.
2. Wash cells again as in step 1.
3. Resuspend cells to a concentration of 1-10 x 10<sup>6</sup>/mL in Annexin V Binding Buffer (1X).
4. Aliquot 100 uL of cell suspension to an individual tube for staining.
5. Add 5 uL of FITC Annexin V conjugate to sample.
6. Add 5 uL of 7-AAD Solution to sample.
7. Gently vortex the cells and incubate at 25°C (room temperature) in the dark for 15 minutes.
8. Add 400 uL of Annexin V Binding Buffer (1X) to each tube.
9. Analyze by flow cytometry promptly.

**NOTE:** It is recommended to prepare a sample with APC Annexin V only and a sample with 7-AAD solution only for proper instrument setup.

**CAUTION:** 7-AAD is a potential carcinogen. Protect skin and eyes by wearing suitable protective clothing, gloves and eye/face protection.