

## Annexin V-FITC/PI Protocol

### Reagents

Propidium Iodide (PI) 1mg/ml stock: [PI]<sub>F</sub> = 2ug/ml  
Annexin V-FITC Jurkat Cells (ATCC TIB-152)  
Etoposide (Sigma Chemical Co., Cat.# E-1383)  
MK-886 (Biomol Research Labs, Inc., Cat.# EI-266)

### Binding Buffer

10mM HEPES, 140mM NaCl, 2.5mM CaCl<sub>2</sub>, pH 7.4

### Optional Binding Buffer (Purdue Cytometry CDROM Vol. #4)

10mM HEPES, 140mM NaCl, 2.5mM CaCl<sub>2</sub>, 5mM KCl, 1mM MgCl<sub>2</sub>, pH 7.4

### Procedure

1. Wash 1X10<sup>6</sup> single cells 2X in cold PBS.
2. Wash cells 2X with Binding Buffer.
3. Re-suspend cells in 100ul Binding Buffer and Annexin V-FITC. (Titer from 0.1ug – 1.0ug).
4. Incubate at room temp. for 10 minutes.
5. Add 400ul Binding Buffer containing 1ul PI.
6. Incubate on ice for 15 minutes. Analyze by flow cytometry within one hour.

Samples that need longer time for transport or any scheduling conflicts can be stored in Binding Buffer only. The Annexin V-FITC and PI can be added just prior to analysis.