7040 Virginia Manor Road
Beltsville, MD 20705, USA
Web: www.abpbio.com;
Email: info@abpbio.com

5X Annexin-binding Buffer
Catalog Number: A041

Table 1. Contents and Storage Information

Material	Amount	Concentration	Storage	Stability
50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	**50 mL**	**5X**	**2-6 °C**	**The product is stable for 1 year when stored as directed.**

Introduction

Apoptosis is a carefully regulated process of cell death that occurs as a normal part of development. Inappropriately regulated apoptosis is implicated in disease states, such as Alzheimer's disease and cancer. Apoptosis is distinguished from necrosis, or accidental cell death, by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.

In normal live cells, phosphatidyl serine (PS) is located on the cytoplasmic surface of the cell membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, thus exposing PS to the external cellular environment. In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages. The human anticoagulant, annexin V, is a 35–36 kDa Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS. The binding of annexin V to PS requires the presence of Ca^{2+}. Under defined salt and calcium conditions, annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.

Experimental Protocols

Note: The assay has been optimized using Jurkat cells treated with camptothecin to induce apoptosis. Some modifications may be required for use with other cell types or other inducing agents.

1. Induce apoptosis in cells using the desired method. Prepare a negative control by incubating cells in the absence of inducing agent.

2. Harvest the cells after the incubation period and wash in cold phosphate-buffered saline (PBS).

3. Prepare 1X annexin-binding buffer. For example, for ~10 assays, add 2 mL 5X annexin-binding buffer to 8 mL deionized water.

4. Re-centrifuge the washed cells (from step 2), discard the supernatant and resuspend the cells in 1X annexin-binding buffer.

Determine the cell density and dilute in 1X annexin-binding buffer to ~1×10^6 cells/mL, preparing a sufficient volume to have 100 µL per assay.

5. Add appropriate amount of annexin V conjugate and dead cell dye to each 100 µL of cell suspension.

6. Incubate the cells at room temperature for 15 minutes in the dark.

7. After the incubation period, add 400 µL 1X annexin-binding buffer, mix gently, and keep the samples on ice.

8. As soon as possible, analyze the stained cells by flow cytometry.