SANTA CRUZ BIOTECHNOLOGY, INC.

Annexin V Apoptosis Detection Kit: sc-4252 AK

BACKGROUND

Apoptotic cells undergo rapid morphological alterations that indicate the progression of cell death. These include changes in the cytoskeleton and plasma membrane, condensation of the cytoplasm and nucleus and internucleosomal cleavage of DNA. An early indicator of apoptosis is the rapid translocation and accumulation of the membrane phospholipid phosphatidylserine (PS) from the cytoplasmic interface to the extracellular surface. This loss of membrane asymmetry can be detected by utilizing the binding properties of Annexin V. Annexin V is a calcium dependent phospholipid binding protein that preferentially binds to negatively charged phospholipids including PS. Cells progressing through apoptosis can be monitored according to their Annexin V and propidium iodide staining pattern. Early apoptotic cells will bind Annexin V but are not sensitive to intracellular staining with propidium iodide (PI). As cells progress through apoptosis the integrity of the plasma membrane is lost, allowing PI to penetrate and label the cells with a strong yellow-red fluorescence.

The Annexin V apoptosis detection kit includes the reagents required for identifying a population of cells that have initiated apoptosis using a simple staining procedure and analysis by fluorescence microscopy or flow cytometry. Analysis of samples can be done on live cells and does not require cell fixation. Normal viable cells in culture will stain negative for Annexin V FITC and negative for PI. Cells that are induced to undergo apoptosis will stain positive for Annexin V FITC and negative for PI as early as 1 hour after stimulation. Both cells in later stages of apoptosis and necrotic cells will stain positive for Annexin V FITC and PI.

REFERENCES

1. Cohen, J.J., Duke, R.C., Fadok, V.A. and Sellins, K.S. 1992. Apoptosis and programmed cell death in immunity. Cell 10: 267-293.
2. Ellis, R.E., Yuan, J.Y. and Horvitz, H.R. 1991. Mechanisms and functions of cell death. Ann. Rev. Cell Biol. 7: 663-698.
3. Chan, A., Reiter, R., Wiese, S., Fertig, G. and Gold, R. 1998. Plasma membrane phospholipid asymmetry precedes DNA fragmentation in different apoptotic cell models. Histochem. Cell Biol. 110: 553-558.

PRODUCT

The Annexin V apoptosis detection kit (sc-4252 AK) contains 50 µg of Annexin V FITC in 250 µl buffer, 5 ml of 10x Assay Buffer and 2 ml of Propidium Iodide at 50 µg/ ml in PBS. Sufficient reagent for 100 tests, assuming 0.5 µg of Annexin V FITC is used per sample. FITC-conjugated Annexin V is also available individually, provided at 50 µg/250 µl buffer: sc-4252 FITC. Recombinant, unlabeled Annexin V blocking protein is available separately, provided at 50 µg/100 µl buffer: sc-4252 BL.

STORAGE

Store at 4° C, **DO NOT FREEZE**. Stable for one year from the date of shipment. Non-hazardous. No MSDS required.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

PREPARATION OF SOLUTIONS

- 1x Assay Buffer: dilute 1 part 10x Assay Buffer in 9 parts distilled H_2O. Store at 4° C.
- 1x Phosphate Buffered Saline: 9.1 mM dibasic sodium phosphate, 1.7 mM monobasic sodium phosphate and 150 mM NaCl. Adjust to pH 7.4 with NaOH. Sterile filter and store at 4–22° C.

ANNEXIN V FITC STAINING PROCEDURES

A. Staining Non-adherent Cells: for analysis by fluorescence microscopy or flow cytometry

1. Induce apoptosis according to desired method.
2. Collect cells by low speed centrifugation at 1500 rpm for 5 minutes. Wash cell pellet twice with cold PBS and resuspend cell pellet in 1x Assay Buffer at a concentration of 1 x 10^6 cells/ml.
3. Transfer 100 µl aliquot of cells (1 x 10^5 cells) to a 5 ml culture tube.
4. To cell samples add 0.5–5 µl (0.1–1 µg) of Annexin V FITC and 10 µl of Propidium Iodide (PI staining is optional) per 100 µl cell sample. Recommended negative controls for flow cytometry include:

   A) no Annexin V FITC and no PI

   B) Annexin V FITC alone

   C) PI alone.
5. Vortex samples gently and incubate for 15 minutes at room temperature in the dark.
6. For fluorescence microscopy detection, wash cell pellet once with PBS (optional).
7. Analyze samples immediately by either fluorescence microscopy or flow cytometry:

- For detection by fluorescence microscopy: Place cell suspension on glass slide. Cover with glass coverslip. Observe cells under fluorescent microscope using a dual filter set for FITC and rhodamine.
- For detection by flow cytometry: Add 400 µl of 1x Assay Buffer. Analyze samples using a single laser emitting light at 488 nm for FITC.

B. Staining Adherent Cells

- **For detection by fluorescence microscopy:**

1. Grow adherent cells on chamber slides at a density of 0.5–1.0 x 10^5 cells/well.
2. Induce apoptosis according to desired method.
3. Rinse cells with PBS. Wash cells once with 500 µl of 1x Assay Buffer per well.
4. To each well add 100–500 µl of 1x Assay Buffer, using adequate volumes to sufficiently cover cells.
5. Add 0.5–5 µl (0.1–1 µg) of Annexin V FITC and 10 µl of Propidium Iodide (PI staining is optional) per 100 µl Assay Buffer used.
6. Incubate for 15 minutes at room temperature in the dark.

7. Wash cells once with PBS (optional).
8. Cover with glass coverslip and visualize under a fluorescence microscope using a dual filter set for FITC and rhodamine.

• For detection by flow cytometry:

1. Induce apoptosis according to desired method.
2. Trypsinize cells and transfer cells to a 15 ml conical tube. Pellet cells by low speed centrifugation at 1500 rpm for 5 minutes. Gently wash cells in media containing serum. Wash cells once with PBS and resuspend pellet in 1x Assay Buffer at a concentration of 1 x 10^6 cells/ml.
3. Follow steps 3–6 from protocol for non-adherent cells.

RECOMBINANT ANNEXIN V BLOCKING PROCEDURE

A. Staining Non-adherent Cells

1. Induce apoptosis and collect cells as described in Annexin V FITC Staining Procedure for non-adherent cells (steps 1–2).
2. Transfer 100 µl aliquot of cells (1 x 10^5 cells) to a 5 ml culture tube.
3. Add 5–15 µg of purified recombinant Annexin V (sc-4252 BL). The amount of recombinant Annexin V necessary to saturate the binding sites should be determined by titration as it may vary according to cell type.
4. Vortex samples gently and incubate for 15 minutes at room temperature.
5. Proceed with Annexin V FITC staining procedure for non-adherent cells (steps 4–7).

B. Staining Adherent Cells

1. Induce apoptosis and prepare cells as described in Annexin V FITC Staining Procedure for adherent cells, according to preferred detection method (steps 1–2).
2. For detection by flow cytometry: proceed with steps 2–5 of Blocking Procedure for non-adherent cells. For detection by fluorescence microscopy: rinse cells on slides once with 500 µl of 1x Assay Buffer; proceed as follows.
3. To each well add 100–500 µl of 1x Assay Buffer, using adequate volumes to sufficiently cover cells.
4. Add 5–15 µg of purified recombinant Annexin V (sc-4252 BL) to each well. The amount of recombinant Annexin V necessary to saturate the binding sites should be determined by titration as it may vary according to cell type.
5. Incubate for 15 minutes at room temperature.
6. Proceed with Annexin V FITC staining procedure for adherent cells, for detection by fluorescence microscopy (steps 5–8).