Annexin V-Cy3/SYTOX Green Apoptosis Kit

Introduction

Annexin V is a cellular protein, which plays important roles in the inhibition of the activity of phospholipase A1 and blood coagulation by competing for phosphatidylserine (PS) binding sites with prothrombin. Annexin V has a high affinity to PS and is used as a probe to detect cells that have expressed PS on the cell surface. After initiating apoptosis, cells translocate phosphatidylserine (PS) from the inner face of the plasma membrane to the cell surface soon, which can be easily detected by Annexin V.

Annexin V-Cy3/SYTOX Green Apoptosis Kit uses a fluorescent conjugate of Annexin V that can easily detect PS on the cell surface after initiating apoptosis. The one-step staining procedure needs only 10-20 minutes. The result can be analyzed by fluorescence microscopy or by flow cytometry. The Annexin V-Cy3/SYTOX Green Apoptosis Kit can differentiate apoptosis vs necrosis when performing both Annexin V-Cy3 and SYTOX Green staining.

Components and Storage

Components	K1140-20 20 Assays	K1140-50 50 Assays	K1140-100 100 Assays
Annexin V-Cy3	100 μL	250 μL	500 μL
1X Binding Buffer	10 mL	25 mL	50 mL
SYTOX Green	20 μL	50 μL	100 μL

Please store SYTOX Green at -20°C, other components at 2-8°C and protect it from long exposure to light. Stable for 6 months.

Protocol

1. Incubation of Cells with Annexin V-Cy3 and SYTOX Green

1) Induction of apoptosis by desired method.
2) Collect cells.

   For suspension cells, 300×g centrifugation for 5 min, and the supernatant of the medium was discarded.

   For adherent cells, try to use EDTA-Free trypsin to digest cells, 300×g centrifugation for 5 min, and the supernatant was discarded.
3) Wash cells with precooled PBS and collect 1-5×10^5 cells.

4) Resuspend cells in 500 µL of 1X Binding Buffer.

5) Add 5 µL of Annexin V-Cy3 and 1 µL of SYTOX Green (Please remove SYTOX green from - 20℃ for thawing before experiment). Incubate at room temperature for 10-20 min in the dark.

6) Proceed to 2 or 3 below depending on method of analysis.

2. Quantification by Flow Cytometry

Analyze Annexin V-Cy3 binding by flow cytometry (Ex = 543 nm, Em = 570 nm) using the phycoerythrin emission signal detector (usually FL2), SYTOX Green-DNA was detected by the FL1 signal detector with a maximum excitation of 504 nm and an emission maximum of 523 nm.

3. Detection by Fluorescence Microscopy

1) Place the cell suspension on a glass slide. Cover the cells with a glass coverslip.

***Note**

For analyzing adherent cells, grow cells directly on a coverslip. Following incubation (1.5), invert coverslip on a glass slide and visualize cells. The cells can also be washed and fixed in 2% formaldehyde before visualization.

Cells must be incubated with Annexin V-Cy3 before fixation since any cell membrane disruption can cause nonspecific binding of Annexin V to PS on the inner surface of the cell membrane.

2) Observe the cells under a fluorescence microscope using a rhodamine filter. Cells that have bound Annexin V-Cy3 will show red staining in the plasma membrane. Cells that have lost membrane integrity will show green staining (SYTOX Green) throughout the nucleus and a halo of red staining (Cy3) on the cell surface (plasma membrane).

***Note**

In general, microscopy based assays recommend appropriately elevated concentrations of Annexin V relative to the amount used for flow cytometry.

Troubleshooting

Problems	Cause	Solution
High Background	▪ Cell density is higher than recommended ▪ Increased volumes of components added ▪ Incubation of cell samples for extended periods ▪ Use of extremely confluent cells ▪ Contaminated cells	▪ Refer to datasheet and use the suggested cell number ▪ Use calibrated pipettes accurately ▪ Refer to datasheets and incubate for exact times ▪ Perform assay when cells are at 80-95% confluency ▪ Check for bacteria/ yeast/ mycoplasma contamination
Lower signal	▪ Washing cells with PBS before/after fixation	▪ Always use binding buffer for washing cells

levels	(adherent cells) ▪ Cells did not initiate apoptosis ▪ Very few cells used for analysis ▪ Incorrect setting of the equipment used to read samples ▪ Use of expired kit or improperly stored reagents	▪ Determine the time-point for initiation of apoptosis after induction (time-course experiment) ▪ Refer to data sheet for appropriate cell number ▪ Refer to datasheet and use the recommended filter setting ▪ Always check the expiry date and store the components appropriately
Erratic results	▪ Uneven number of cells seeded in the wells ▪ Adherent cells dislodged at the time of experiment ▪ Incorrect incubation times or temperatures ▪ Incorrect volumes used ▪ Increased or random staining observed in adherent cells	▪ Seed only healthy cells (correct passage number) ▪ Perform experiment gently and in duplicates or triplicates for each treatment ▪ Refer to datasheet & verify correct incubation times and temperatures ▪ Use calibrated pipettes and aliquot correctly ▪ Always stain cells with Annexin before fixation (makes cell membrane leaky)

***Note:**

The most probable cause is listed under each section. Causes may overlap with other sections.

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