Annexin V apoptosis detection Kit (PE)

(100 assays)

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CAT No. ZX-11100-100-B

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Application:

Detection of early/middle stages of apoptosis

Differentiation of apoptosis from necrosis

!!! Caution: This product is for Research Use Only. Not for *in-vitro* Diagnostics !!!

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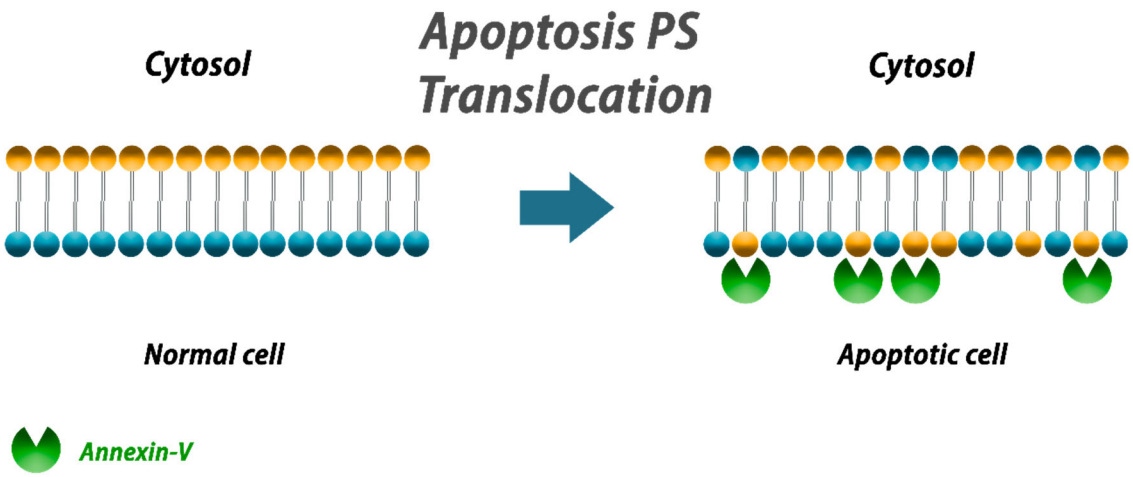
Please read this insert completely prior to using the product.

Background

Apoptosis is the process of programmed cell death, which occurs as a normal physiologic process and plays an important role in embryonic development and maintenance of tissue homeostasis. It is characterized by special morphologic features, including asymmetric loss of plasma membrane and attachment- as one of the earliest features, condensation of the nuclear chromatin, internucleosomal cleavage of DNA and cell membrane blebbing. During apoptosis, the membrane phospholipid phosphatidylserines (PS) are translocated from the inner to the outer layer of the plasma membrane and exposed to the external cellular environment.

Annexins are a family of calcium-dependent phospholipid-binding proteins that can bind to PS. Fluorophoe-conjugated Annexin V is commonly used to visualize apoptosis via detection of externalized phosphatidylserine residues on the outer layer of plasma membrane in apoptotic cells. Binding of Annexin V and PS occurs in the presence of Calcium ion (Ca^{2+}). Upon the attachment of apoptotic cells to labelled Annexin V, apoptosis can be measured using fluorescent microscopy or flow-cytometry.



In contrast to apoptosis, necrosis is characterized by the loss of membrane integrity which allows for the staining of necrotic cells with specific membrane-impermeant nucleic acid dyes such as propidium iodide (PI). Therefore, one way to differentiate apoptosis from necrosis is to demonstrate the membrane integrity of apoptotic cells by the exclusion of these nucleic acid dyes.

Intended use

Detection of early/middle stages of apoptosis

Differentiation of apoptosis from necrosis

Materials supplied in the Kit

Component	*Quantity*
Labeled Annexin V (PE)	500 µL
Propidium Iodide (PI)	1 mL
Binding Buffer Conc. (10 X)	50 mL

Storage instruction

All reagents should be stored at 4° C, protected from light, until the expiration date of the kit.

Materials required but not supplied

Precision pipettes and disposable filter pipette tips

Distilled water (dH_2O)

Sterile test tubes

Precautions

This kit should only be used by qualified personnel who have had laboratory safety instruction. The complete insert should be read and understood before attempting to use the product.

General remarks

- The instruction must be strictly followed.
- Pipette tips should not be used more than once to avoid cross contamination.
- Reagents of different batches should not be mixed or used after their expiration dates.
- **Propidium Iodide** is a suspected carcinogen; contact with eyes, skin, and mucous membranes must be avoided. Always wear proper protective clothing and gloves when handling the solution.

Reagent preparation

i. **Binding Buffer:** Prepare a 1:10 dilution of Binding Buffer Concentrate with dH_2O (1 part Binding Buffer Conc. with 9 parts dH_2O), and mix well. Binding Buffer must be kept on ice.

Assay Procedure

1. Centrifuge and collect the cells (1-5 x 10^6 cells).
2. Wash cells with ice-cold culture medium or PBS.
3. Resuspend cells in 1 mL Binding Buffer (1-5 x 10^6 cells/mL).
4. Transfer 500 µl of cell suspension in a test tube.
5. Add 5 µl of labeled- Annexin V to the test tube.
6. Add 10 µl of Propidium Iodide Solution to the test tube.
7. Gently vortex the cells and incubate for 15 min at room temperature (25°C), protected from light.
8. Wash cells with 1 X Binding Buffer and resuspend in 400 µl of 1 X Binding Buffer.
9. Analyze by flow cytometry with proper machine settings.