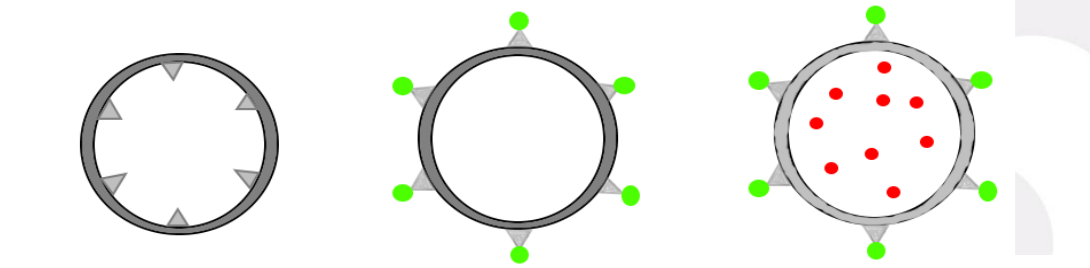
Xpert Annexin V-FITC Apoptosis Detection Assay

#GTC21.0100 (100 assays) | GTC21s (trial size)
(FOR RESEARCH ONLY)

Product:
The Xpert Annexin V-FITC Apoptosis Detection Assay offers a simple, fast and reliable method for the fluorescent detection of apoptotic cells and quantitative determination by flow cytometry. This assay is based on binding of FITC-labeled Annexin V to phosphatidylserine sites (PS) present on the membrane surface of apoptotic cells combined with the binding of propidium iodide (PI) to cellular DNA in necrotic cells in which cell membrane integrity has been completely compromised. This allows to distinguish between viable cells, early apoptotic cells and necrotic cells.

Apoptosis is a gradual process leading to cell death, characterized by specific morphologic features. The translocation of PS from the inner to the outer leaflet of the plasma membrane, hence exposing PS to the external cellular environment, is one of the earliest phenomena of apoptosis, preceding loss of membrane integrity that occurs in later stages of apoptosis or during necrosis. Therefore, when using a combination of green fluorescent FITC-labeled Annexin V (Annexin A5) and red fluorescent PI, viable cells are negative for both, whereas early apoptotic cells are Annexin V-positive, as Annexin V binds strongly to PS, even when labeled with FITC, but PI-negative (as intact membranes are impermeable to PI). Cells entering later stages of apoptosis or necrosis will be both Annexin V and PI-positive.



1. Viable Cells
▲ = phosphatidylserine

2. Early-Stage Apoptosis
● = Annexin V-FITC

3. Late-Stage Apoptosis/Necrosis
● = Propidium Iodide

Albeit that this assay does not distinguish between cells that have died as a result of apoptosis and cells that have suffered another necrotic pathway, by measuring apoptosis over time, cells can often be followed from Annexin V-negative/PI-negative via Annexin V-positive/PI-negative to Annexin V-positive/PI-positive. Encountering all these three phenotypes at the same time within a cell population, or observing a shift through these three stages within a cell population over time, is thus a strong indicator of apoptosis.

Contents:

Product (100 assays)	GCT21.0100
FITC-labeled Annexin V	500 µl
Binding Buffer (10X)	50 ml
Propidium Iodide	1 ml

Quantity: #GTC20.0025 contains 25 ml of Resazurin solution, sufficient for 2,500 assays (96-well format)
#GTC20s is a trial sample sufficient for 200 assays (96-well format)

Storage: All components should be stored at +4ºC. The product is stable for at least 2 years. Do not freeze and protect from prolonged exposure to light.

Prior to use:

Dilute 10x concentrated Binding Buffer with ultrapure water to 1x and keep on ice. For the first washing step, keep some culture medium on ice. It is recommended to include a positive control for apoptosis.

Flow cytometry

1. Harvest cells of interest by centrifugation. If working with adherent cells, first detach cells by trypsinizing or with Accutase®

2. Gently wash cells with ice-cold sterile PBS (or ice-cold culture medium) and transfer to a 5-ml sterile cell culture test tube. Wash the pellet again with ice-cold sterile PBS and resuspend in 500µl of 1x ice-cold Binding Buffer at a concentration of 10^5-10^6 cells/ml

3. Add 5µl of FITC-labeled Annexin V and 10µl of Propidium Iodide

4. Gently vortex and incubate for at room temperature (25°C) in the dark for 15 min

5. Gently wash cells with 1x ice-cold Binding Buffer and resuspend in 400µl of 1x Binding Buffer, and analyze within an hour by flow cytometry

6. Analyze cell population by flow cytometry:
   For FITC-labeled Annexin V binding to PS use the FITC signal detector (excitation λ=488nm, emission λ=530nm) (usually FL1)
   For Propidium Iodide binding to DNA use the PE signal detector (excitation λ=535nm, emission λ=617nm) (usually FL2)

Fluorescent microscopy (non-adherent cells)

1. Harvest cells of interest by centrifugation and wash cells with ice-cold sterile PBS (or ice-cold culture medium), transfer to a 5-ml sterile cell culture test tube, and wash the pellet again with ice-cold PBS.

2. Resuspend in 100µl of 1x ice-cold Binding Buffer at a concentration of 10^6 cells/ml (so that 100µl contains 10^5 cells)

3. Add 5µl of FITC-labeled Annexin V and 10µl of Propidium Iodide

4. Gently vortex and then incubate for at room temperature (25°C) in the dark for 15 min

5. Add the cells to a glass microscope slide, then coverslip and image Annexin V FITC with a FITC filter set and Propidium Iodide with a rhodamine filter set.

Fluorescent microscopy (adherent cells)

1. Culture cells on 22mm coverslips to a density of 1x 10^5 cells/coverslip.

2. Wash cells twice with ice-cold sterile PBS and incubate with 500µl of 1x ice-cold Binding Buffer

3. Add 5µl of FITC-labeled Annexin V and 10µl of Propidium Iodide and gently rock at room temperature (25°C) in the dark for 15 min.

4. Image cells directly using FITC filter set and rhodamine filter set or wash in ice-cold binding buffer and fix with 2% formaldehyde.