molecular probes®
by life technologies™

Tali® Apoptosis Kit – Annexin V Alexa Fluor® 488 and Propidium Iodide

for use with Tali® Assay: Apoptosis

Catalog no. A10788

Table 1 Contents and storage

Material	Amount	Composition	Storage	Stability
Annexin V Alexa Fluor® 488 (Component A)	500 µL	Solution in 25 mM HEPES, 140 mM NaCl, 1 mM EDTA, pH 7.4, 0.1% bovine serum albumin (BSA)	• 2–8°C • Protect from light • Do not freeze	When stored as directed, the product is stable for at least 6 months.
Tali® Propidium Iodide (PI) (Component B)	100 µL	100 µg/mL in water	• 2–30°C	
5X Annexin Binding Buffer (ABB) (Component C)	25 mL	50 mM HEPES, 700 mM NaCl, 12.5 mM $CaCl_2$, pH 7.4	• 2–8°C • Protect from light	
Number of assays: Sufficient material is supplied for 100 assays of 100 µL each.				
Approximate fluorescence excitation/emission maxima: Annexin V Alexa Fluor® 488: 488/499 in nm; Propidium iodide: 535/617 in nm, bound to DNA.				

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 by characteristic morphological and biochemical changes, including compaction and fragmentation of the nuclear chromatin, shrinkage of the cytoplasm, and loss of membrane asymmetry.[1–5] 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.[6] In leukocyte apoptosis, PS on the outer surface of the cell marks the cell for recognition and phagocytosis by macrophages.[7,8]

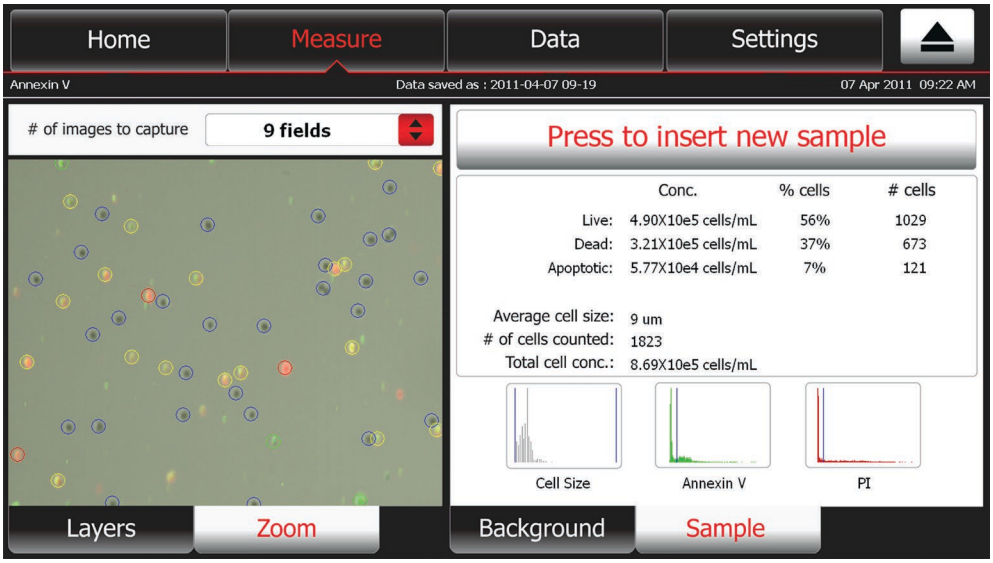
The human anticoagulant, annexin V, is a 35–36 kDa Ca^{2+}-dependent phospholipid-binding protein that has a high affinity for PS.[9] Annexin V labeled with a fluorophore or biotin can identify apoptotic cells by binding to PS exposed on the outer leaflet.[10]

Propridium Iodide (PI) is a cell-impermeant and fluorogenic DNA-binding dye used foridentifying necrotic cells. PI is impermeant to live cells, but easily enters dead cells where it binds to nucleic acids and becomes fluorescent.

After a cell population is stained using the Tali® Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 and Propidium Iodide, apoptotic cells show green fluorescence, dead cells show red and green fluorescence (observed as yellow), and live cells show little or no fluorescence. The Tali® Image-Based Cytometer captures up to 20 images (i.e., fields of view) of the stained sample, automatically analyzes the images with sophisticated digital image-based cell counting and fluorescence detection algorithms, and presents the results of the analysis in the Sample tab (Figure 1, below). The data from the analysis, including the image files, can then be downloaded to a USB flash drive immediately after the assay and transferred to a computer for sample comparisons.

The Tali® Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 is compatible with a most suspension cell lines. For a list of the cell lines with which the Tali® Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 has been validated, refer to **www.lifetechnologies.com/tali**.

Figure 1 Example of a Tali® Apoptosis Assay using Tali® Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 and Propidium Iodide. The Sample tab shows the concentration, the relative proportion, and the number of live, dead, and apoptotic cells. The image window shows the captured fields of view, where the apoptotic cells with green fluorescence are clearly distinguishable from dead cells that fluoresce red and live cells that do not fluoresce. For detailed instructions on using the Tali® Image-Based Cytometer, refer to the user guide supplied in the Tali® Image-Based Cytometer USB Drive. The user guide is also available for downloading at www.lifetechnologies.com/tali.



Before You Begin

Materials Recommended but Not Provided

Tali® Cellular Analysis Slides (Cat. nos. T10794, T10795)

Caution

Propidium iodide is a potential mutagen; use appropriate precautions when handling this reagent.

Experimental Protocol

Follow the instructions below to perform the Tali® Apoptosis Assay using the Tali® Apoptosis Assay Kit–Annexin V Alexa Fluor® 488 and Propidium Iodide. For detailed instructions on using the Tali® Image-Based Cytometer, refer to the user guide supplied in the Tali® Image-Based Cytometer USB Drive. The user guide is also available for downloading at **www.lifetechnologies.com/tali**. The recommended sample concentration range for the Tali® Image-Based Cytometer is 1×10^5 to 1×10^7 cells/mL; however, the sample concentration does not need to be exact to perform an assay.

1. If required, induce apoptosis in cells using the desired method. Prepare a negative control by incubating the cells in the absence of the apoptosis inducing agent. If your cells exhibit significant autofluorescence, you may additionally prepare a negative control of unstained cells.

2. Prepare 1X Annexin binding buffer by diluting the 5X Annexin binding buffer (ABB, Component C) in deionized water. For example, for 10 assays, add 200 µL of 5X Annexin binding buffer to 800 µL of deionized water.

3. Harvest the cells after the incubation period, centrifuge, and discard the supernatent.

4. Resuspend the cells in 1X Annexin binding buffer, so that there is at least 100 µL of cells per individual assay at a concentration of approximately 5×10^5 to 5×10^6 cells/mL. The concentration of the cells does not need to be exact.

5. To each 100 µL of sample, add 5 µL of Annexin V Alexa Fluor® 488 (Component A). Mix well.

6. Incubate the cell-Annexin V Alexa Fluor® 488 mixture at room temperature in the dark for 20 minutes.

7. Centrifuge the cells and resuspend them in 100 µL of ABB.

8. Add 1 µL of Tali® Propidium Iodide (PI, component B) to each 100 µL sample. Mix well.

9. Incubate the samples at room temperature in the dark for 1–5 minutes.

10. Load 25 µL of the stained cells into a Tali® Cellular Analysis Slide by pipetting the sample at an angle of approximately 80° into the half moon-shaped sample loading area. The sample is loaded into the chamber through capillary action. Take care to avoid forming bubbles in the sample or to cause back splatter.

11. Insert the slide into the slide port of the Tali® Image-Based Cytometer until it stops. Do not forcefully push the slide any further.

12. Touch **Cell Health** on the Home screen of the Tali® Image-Based Cytometer and then touch **Apoptosis** to select the Tali® Apoptosis Assay.

13. Name the sample series, if desired.

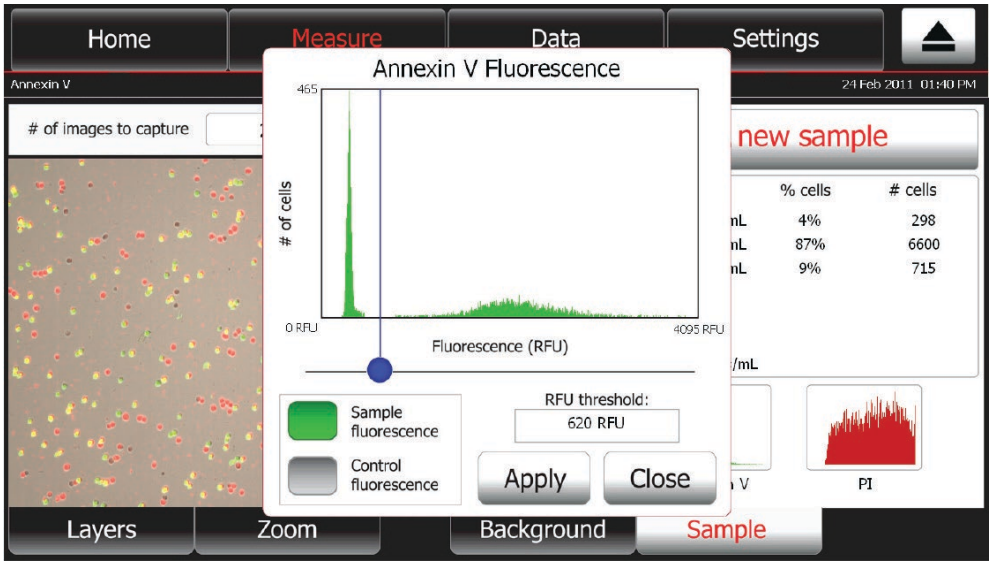
14. Touch **Press to insert new sample**; the slide will automatically be pulled into the instrument.

15. When prompted, focus your cells using the image adjustment (focus) knob on the right side of the instrument.

16. Specify the number of fields of view to capture using the # of images to capture drop-down menu, and then touch **Press to run sample**. The Tali® Image-Based Cytometer will automatically capture and analyze the images of your sample, and present the results of the analysis in the analysis window.

    Note: Biological molecules found within cells fluoresce upon excitation and result in background fluorescence. Because the Tali® Image-Based Cytometer is a highly sensitive instrument, this background fluorescence is detected and displayed as a peak closest to the 0 RFU (relative fluorescence unit) value. To eliminate the background fluorescence from your measurements, adjust the threshold to exclude this peak.

17. On the Sample tab, touch the appropriate **histogram thumbnail** and set the threshold by moving the blue button on the slider bar. The Tali® Image-Based Cytometer automatically re-analyzes the data and updates the results in the Sample tab. The example below shows the threshold pop-up window for the Annexin V fluorescence.



References

1. Immunol Cell Biol 76, 1 (1998); **2.** Cytometry 27, 1 (1997); **3.** J Pharmacol Toxicol Methods 37, 215 (1997); **4.** FASEB J 9, 1277 (1995); **5.** Am J Pathol 146, 3 (1995); **6.** Cytometry 31, 1 (1998); **7.** J Immunol 148, 2207 (1992); **8.** J Immunol 151, 4274 (1993); **9.** J Biol Chem 265, 4923 (1990); **10.** Blood 84, 1415 (1994).

Product List Current prices may be obtained from our website or from our Customer Service Department.

Cat no.	Product Name	Unit Size
A10788	Tali® Apoptosis Kit – Annexin V Alexa Fluor® 488 and Propidium Iodide	1 kit
Related Products		
A10786	Tali® Viability Kit – Dead Cell Red (for use with Tali® Assays: Viability, Green + Red)	100 assays
A10787	Tali® Viability Kit – Dead Cell Green (for use with Tali® Assays: Green, Green + Red)	100 assays
A10794	Tali® Cellular Analysis Slides, 50 slides	1 each
A10795	Tali® Cellular Analysis Slides, 500 slides	1 each
A10798	Tali® Cell Cycle Kit.	50 assays

Purchaser Notification

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