Tali™ Apoptosis Kit - Annexin Alexa Fluor™ 488 and Propidium Iodide

For use with Tali™ Assay: Apoptosis

Catalog Number A10788

Doc. Part No. MP10788 **Pub. No.** MAN0004128 **Rev.** 3.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Product description

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 Ca2+-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 for identifying necrotic cells. PI is impermeant to live cells, but easily enters dead cells where it binds to nucleic acids and becomes fluorescent.

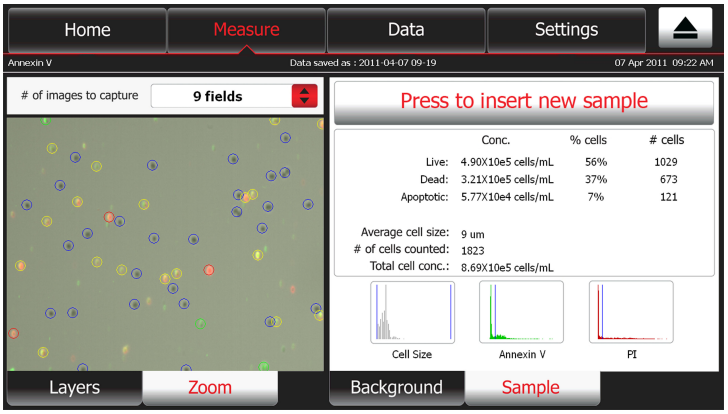


Figure 1 Example of a Tali™ Apoptosis Assay using Tali™ Apoptosis Kit - Annexin Alexa Fluor™ 488 and Propidium Iodide.

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 **thermofisher.com/tali**.

After a cell population is stained using the Tali™ Apoptosis Kit - Annexin 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 (see Figure 1). 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 Kit - Annexin Alexa Fluor™ 488 and Propidium Iodide is compatible with a most suspension cell lines. For a list of the cell lines with which the Tali™ Apoptosis Kit - Annexin Alexa Fluor™ 488 and Propidium Iodide has been validated, refer to **thermofisher.com/tali**.

Contents and storage

Sufficient material is supplied for 100 assays of 100 µL each.

Contents	Amount	Composition	Storage [1]
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°C to 8°C • Protect from light • Do not freeze
Tali™ Propidium Iodide (PI) (Component B)	100 µL	100 µg/mL in water	• 2°C to 30°C
1X Annexin Binding Buffer (ABB) (Component C)	25 mL	10 mM HEPES, 140 mM NaCl, 2.5 mM $CaCl_2$, pH 7.4	• 2°C to 8°C • Protect from light

[1] When stored as directed, the product is stable for at least 6 months.

Table 1 Approximate fluorescence excitation/emission maxima

Components	Maximum Excitation	Maximum Emission
Annexin V Alexa Fluor™ 488	488 nm	499 nm
Propidium Iodide	535 nm	617 nm

Materials required not included

- Tali™ Cellular Analysis Slides (Cat. Nos. T10794 and T10795)

Perform the Tali™ Apoptosis Assay

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 **thermofisher.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. *(Optional)* 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. Harvest the cells after the incubation period, centrifuge, and discard the supernatant.

3. Resuspend the cells in 1X Annexin Binding Buffer (ABB).

   The concentration of the cells does not need to be exact, but it should be at least 100 µL of cells per individual assay at a concentration of approximately 5×10^5–5×10^6 cells/mL.

4. Add 5 µL of Annexin V Alexa Fluor™ 488 (Component A) to each 100 µL of sample, then mix well.

5. Incubate the cell-Annexin V Alexa Fluor™ for 20 minutes mixture at room temperature in the dark.

6. Centrifuge the cells, then resuspend them in 100 µL of ABB.

7. Add 1 µL of Tali™ Propidium Iodide (PI, component B) to each 100 µL sample, then mix well.

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

9. 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.

10. Insert the slide into the slide port of the Tali™ Image-Based Cytometer until it stops.

    Do not forcefully push the slide any further.

11. Touch **Cell Health** on the **Home** screen of the Tali™ Image-Based Cytometer, then touch **Apoptosis** to select the Tali™ Apoptosis Assay.

12. *(Optional)* Name the sample series.

13. Touch **Press to insert new sample**.

    The slide will automatically be pulled into the instrument.

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

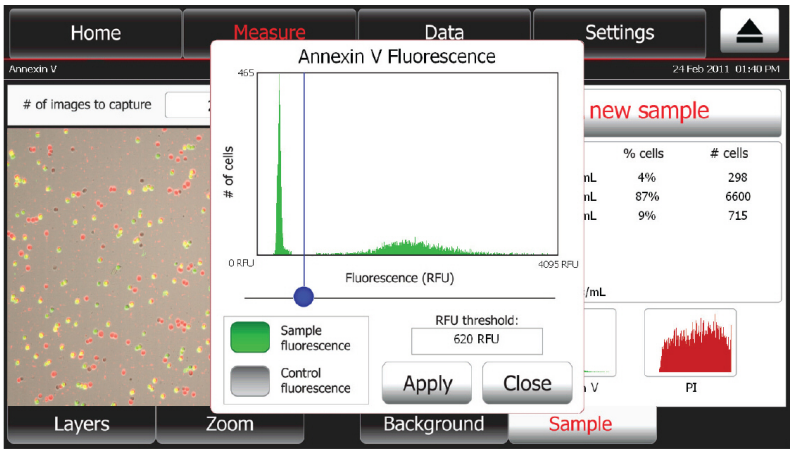
15. Specify the number of fields of view to capture using the # of images to capture drop-down menu, then touch **Press to run sample**.

    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.

    The Tali™ Image-Based Cytometer will automatically capture and analyze the images of your sample, then present the results of the analysis in the analysis window.

16. On the Sample tab, touch the appropriate **histogram thumbnail**, then set the threshold by moving the blue button on the slider bar.

    The following example shows the threshold pop-up window for the Annexin V fluorescence.



The Tali™ Image-Based Cytometer automatically re-analyzes the data and updates the results in the Sample tab.

Related products

Unless otherwise indicated, all materials are available through **thermofisher.com**.

Products	Size	Cat. Nos.
Tali™ Viability Kit - Dead Cell Red (for use with Tali™ Assays: Viability, Green + Red)	100 assays	A10786
Tali™ Viability Kit - Dead Cell Green (for use with Tali™ Assays: Viability, Green + Red)	100 assays	A10787
Tali™ Cellular Analysis Slides	50 slides	A10794
Tali™ Cellular Analysis Slides	500 slides	A10795
Tali™ Cell Cycle Kit	50 assays	A10798

References

1. Immunol Cell Biol 76, 1 (1998)
2. Cytometry 27, 1 (1997)
3. J Pharmacol Toxicol Methods 37, 215 (1997) 4923 (1990); 10. Blood 84, 1415 (1994).
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)

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Manufacturer: Life Technologies Corporation | 29851 Willow Creek | Eugene, OR 97402

The information in this guide is subject to change without notice.