

Product Information

YF®488-Annexin V and RedNucleus II Apoptosis Kit

Catalog Number: Y6102

Product Size: 50 T, 100 T

Contents:

Component	50 T	100 T
A. 1×Annexin V Binding buffer	50 mL	50 mL×2
B. YF®488 -Annexin V	250 µL	500 µL
C. RedNucleus II	500 µL	1 mL

Storage

Store at 4°C and protect from light. When stored as directed, product is stable for at least 6 months. Do not freeze.

Spectral Characteristics

YF®488-Annexin V: Abs/Em = 490 /515 nm

RedNucleus II: Abs/Em = 635 /695 nm (with DNA)

Description

YF®488 and RedNucleus II Apoptosis Kit provides a convenient method to make a distinction between apoptotic (green) and necrotic (red) cells within the same cell population by flow cytometry or fluorescence microscopy.

Fluorescent conjugates of Annexin V can be used to label apoptotic cells. Human anticoagulant Annexin V is a 35-36 kilodalton, Ca²⁺-dependent phospholipid-binding protein with high affinity for phosphatidylserine (PS). In normal viable cells, PS is located on the inner leaflet of the cytoplasmic membrane. However, in apoptotic cells, PS is translocated from the inner to the outer leaflet of the plasma membrane, where it is available for binding to fluorescently labeled Annexin V, which can be detected by fluorescence microscopy or flow cytometry.

RedNucleus II provided in this kit is a far red fluorescence dye, belonging to anthraquinone compounds, which can not penetrate the intact cell membrane of living cells and early apoptotic cells, and is impermeable, but can rapidly stain nucleus/dsDNA in dead and permeable cells. RedNucleus II is an ideal substitute of propidium iodide (PI) and 7-AAD. It can be used in multicolor analysis with FITC, PE and purple fluorescent dyes because it is not excited by ultraviolet and overlapped with PE/PE homologues without compensation controls. When combined with YF®488-Annexin V, RedNucleus II was excluded from living cells and early apoptotic cells, while late apoptotic cells and dead cells were stained with YF®488-Annexin V and RedNucleus II at the same time.

There is no spectral cross between YF®488 and RedNucleus II, so there is no need to do compensation control for cells without spontaneous fluorescence, self-contained RFP or apoptosis cells induced by adriamycin.

Protocol

These protocols were optimized using Jurkat cells treated with staurosporine to induce apoptosis. Additional assay

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Tel: 0512-88965152
Web: www.useverbright.com/

optimization may be required for use with other inducing agents or other cell types.

Staining protocol for flow cytometry

1. Induce apoptosis. Include an untreated cell sample as negative control. Also include samples for single-stained controls if compensation is required.
2. Collecting cells after treatment by centrifugation and wash with PBS.

Note: If you prefer not to wash cells, staining can be performed in cell culture medium with serum instead of Annexin Binding Buffer, but the concentration of Annexin V may require optimization.

3. Centrifuge cells again, discard supernatant and resuspend cells for 5×10^6 to 10^7 cells per mL with $1 \times$ Binding Buffer.
4. Aliquot cells into flow cytometry tubes at 100 μ L/tube.
5. Add 4-5 μ L YF[®]488-Annexin V and 5 μ L RedNucleus II to each tube.

Note: we recommend to prepare three additional tubes of apoptosis induced cells, one tube without dye, to regulate the voltage of FSC and SSC, and the other two tubes with only one dye (YF[®]488 or RedNucleus II) for channel voltage regulation.

6. Incubate at room temperature for 10-15 minutes in the dark. The incubation can be carried out on ice to arrest the apoptotic process if desired.
7. Add 400 μ L $1 \times$ Binding Buffer to each tube and analyze by flow cytometry within 30 minutes. Use 488 nm excitation and measure fluorescence emission near 530 nm (YF[®]488 channel), and use 633 nm excitation and measure fluorescence emission

near 695 nm (RedNucleus II channel).

Staining protocol for fluorescence microscopy

For cells in suspension, follow the staining protocol for flow cytometry.

1. Grow cells on coverslips or chamber slides.
2. Induce apoptosis. Include an untreated cell sample as a negative control.
3. Wash cells with PBS.

Note: If you prefer to not wash cells, staining and imaging can be performed in cell culture medium with serum instead of Annexin Binding Buffer, but the concentration of Annexin V may require optimization.

4. Add 5-25 μ L of YF[®]488-Annexin V and 5 μ L of RedNucleus II into every 100 μ L Annexin Binding Buffer.

Note: The optimal concentration may need to be determined empirically.

5. Add enough staining solution to completely cover the cells, and incubate at room temperature for 15-30 minutes in the dark. Incubation can be carried out on ice to arrest the apoptotic process if desired, but staining time should be at least 30 min.
6. Wash cells with $1 \times$ Binding Buffer.
7. Mount coverslips onto slides with a drop of $1 \times$ Binding Buffer. For cells on chamber slides, add enough $1 \times$ Binding Buffer to completely cover cells.
8. Using appropriate filters to image. YF[®]488 Annexin V can be imaged with FITC filter, while RedNucleus II can be imaged with Cy 5.5 filter.

