LEADGENE
LEADGENE BIOMEDICAL, INC.

Product Information & Manual

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Annexin V-FAM + PI Apoptosis Detection Reagent

Catalogue Number LDG0002RB

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO

Store at 2-8℃

LEADGENE BIOMEDICAL, INC.
No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan
TEL: +886-6-2536677 • FAX: +886-6-2531536
Email: info@leadgene.com.tw • www.leadgenebio.com

Leadgene® Annexin V-FAM + PI Apoptosis Detection Reagent

1. Introduction

Apoptosis is a gradually orchestrated process of biochemical reactions of a cell from an organism. It can be triggered by many stimuli, including infection, hypoxia, ischemia, nutrient removal, toxins, heat, radiation, drugs, chemicals, and disease. Consequently, these stresses alter the morphology of a cell, including cell shrinkage, nuclear and cytoplasmic condensation, chromatin fragmentation, membrane blebbing, and apoptotic body formation.

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2. Test principle

Leadgene fluorescent dye (FAM) conjugated Annexin V is highly purified product. During early apoptosis, cells will translocate membrane phosphatidylserine (PS) from the inner face of the membrane to the cell surface. Propidium iodide (PI) is a common fluorescent dye to detect DNA. It can be used in flow cytometry to evaluate the cell cycle and cell viability during apoptosis. The product can be used in one-step staining procedure without wash step within 20 minutes.

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3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity	Reconstitution
25 reactions	**Annexin V-FAM** 1 vial (0.125 mL)	**Ready** for use
	PI 1 vial (0.125 mL)	**Ready** for use
	10X Binding Buffer 1 vial (2 mL)	**Dilute** 10 x with distilled water (see reagent preparation, section 5)
50 reactions	**Annexin V-FAM** 1 vial (0.25 mL)	**Ready** for use
	PI 1 vial (0.25 mL)	**Ready** for use
	10X Binding Buffer 2 vials (2 mL)	**Dilute** 10 x with distilled water (see reagent preparation, section 5)
100 reactions	**Annexin V-FAM** 1 vial (0.5 mL)	**Ready** for use
	PI 1 vial (0.5 mL)	**Ready** for use
	10X Binding Buffer 3 vials (2 mL)	**Dilute** 10 x with distilled water (see reagent preparation, section 5)

4. Materials required but not provided

(1) Flow tube
(2) 100 µL to 1000 µL adjustable single-channel micropipette with disposable tips
(3) 1 µL to 10 µL adjustable single-channel micropipette with disposable tips
(4) Phosphate-buffered saline (PBS)
(5) Disposable microcentrifuge tubes
(6) Centrifugation machine
(7) Timer
(8) Disposable gloves
(9) Discard container for bio-medical waste
(10) High quality distilled water

5. Reagent preparation

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The working reagents should be prepared with adequate volume and discarded at the end of the day.

- **Working binding buffer (1 X):** Dilute 1 volume of **10 X binding buffer** with 9 volumes of distilled water and homogenize by using micropipette.

6. Storage and expiration date of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at 2-8℃ in the dark.
- The remaining reagents should be immediately returned to 2-8℃ in the dark after used
- Alterations in physical appearance of kit components may indicate instability or deterioration.
- All reagents are stable for one year under proper storage conditions.

7. Procedure

(1) Collect 1-5x10^5 cells in the flow tube by centrifugation.

(2) Wash cells in 2 mL cold phosphate-buffered saline (PBS) and collect by centrifugation.

(3) Re-suspend cell in 500 µL of 1X Binding Buffer.

(4) Add 5 µL of Annexin V-FAM and 5 µL of PI, and gently mix the cells and incubate for 15-20 minutes at RT in the dark.

(5) After incubation, the samples should be kept on ice and perform flow cytometry using filters appropriated for fluorescein (FITC, corresponding to Annexin V-FAM).

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