# RayBio® Annexin V Apoptosis Detection Kit

## (RayBright® Violet 450)

**User Manual Version 1.0**
**Oct 31st, 2018**

**RayBio® Annexin V Apoptosis Detection Kit**
**(RayBright® Violet 450)**

**Catalog numbers: 137-08010-25 (25 tests)**
**137-08010-100 (100 tests)**

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RayBiotech, Inc.

# RayBio® Intracellular Staining Kit

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## I. INTRODUCTION

The RayBiotech Annexin V Detection Kit is a sensitive assay for detection of cell apoptosis by flow cytometry. Annexin V is a cellular protein that specifically binds to phosphatidylserine (PS) sites on cell membrane. In live healthy cells, PS is predominantly localized along the cytosolic side of the plasma membrane. However, during induction of apoptosis or necrosis, PS translocates to the extracellular membrane. If present on the extracellular surface, the PS can be detected by fluorochrome-labeled Annexin V. With the inclusion of RayBiotech's fixable live/dead marker stain, live and dead cells can be discriminated as well. However, unlike other apoptosis assay kits using propidium iodide (PI) or 7-AAD, RayBio Annexin V apoptosis Detection Kit can be combined with immunophenotyping that requires cellular fixing of the cell populations. Combined, the RayBiotech Annexin V Detection kit allows for a fixable live/dead and apoptotic/necrotic staining kit.

## II. KIT CONTENTS

| Components | 137-08010-25 | 137-08010-100 | Part Number |
|---|---|---|---|
| | 25 tests | 100 tests | |
| RayBright® R780 Live (in DMSO, 1:100 dilution for use) | 25 µL | 100 µL | Item A |
| RayBright® V450 Annexin V (5 µL/test) | 125 µL | 500 µL | Item B |
| Annexin V Binding Buffer, 5x (dilute with ddH2O to 1x solution) | 5 mL | 20 mL | Item C |
| Annexin V Co-staining Buffer, 5x (dilute with ddH2O to 1x solution) | 5 mL | 20 mL | Item D |

- Kit can be stored at 4°C, protected from light for up to 3 months
- Make enough Live/Dead dye (Item A), 1x Binding Buffer (Item C), and Co-staining Buffer (Item D) for your assay. Return remaining stock at 4°C.

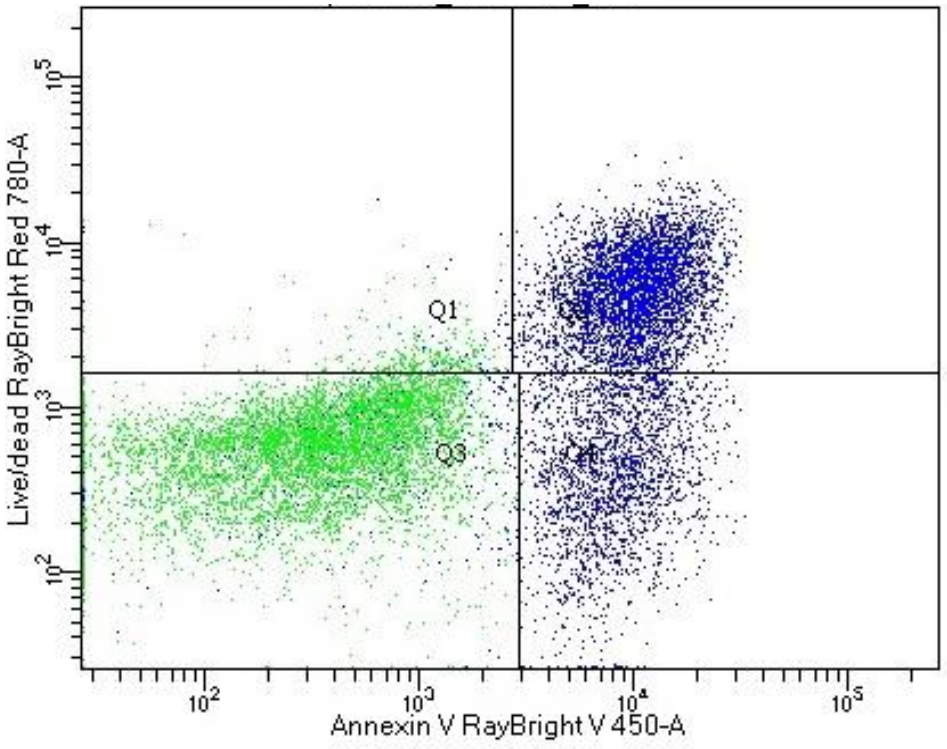
## III. Assay Protocol

1. Transfer cells to a 96-well plate.
2. Wash cells twice with cold PBS, spin down cells at 300g for 10 minutes at 4°C (same centrifuge speed applies to the following steps).
3. Resuspend cells in 100 µL of PBS containing diluted RayBright® 780 live/dead marker (Item A, 1:100 dilution in PBS)
4. Incubate for 20 minutes on ice.
5. Spin down the cells, wash cells twice with 200 µL 1x Annexin V Binding Buffer (Item C)
6. Resuspend cells in 100 µL of 1x annexin V binding buffer, add 5 µL of fluorochrome-conjugated-annexin V (Item B), incubate at room temperature for 15 minutes.
7. Wash the cells once with 200 µL 1x Annexin V Binding Buffer, then resuspend in 400 µL of 1x Annexin V Binding Buffer for analysis, or continue to Steps 8-11 if immunophenotyping is required (optional).
8. **For immunophenotyping**: Resuspend cells with 50 µL 1x Annexin V Co-staining Buffer (Item D) containing Fc Receptor blocking reagents (e.g. RayBright® Human or Mouse FcR Block), incubate for 5 minutes.

9. Add 50 µL of Annexin V Co-staining Buffer containing your cocktail of fluorochrome-conjugated antibodies to the above cells (total volume of 100 µL) and incubate for 25 minutes at 4°C.
10. Wash the cells with 200 µL of 1x Annexin V Co-staining Buffer and spin down the cells.
11. Add 400 µL of 1x Annexin V Co-staining Buffer to each well, keep samples on ice and analyze by flow Cytometry.

Representative Data:

- One million human PBMCs were stained with RayBright® Live R780 and RayBright® V450 Annexin V in 1x Annexin V Binding Buffer per the above protocol.




## IV. Storage and Stability

Store kit at 4°C, protected from light for up to three months for full functionality

This product is for research use only.