

eBioscience™ Annexin V-Biotin Apoptosis Detection Kit

Catalog Numbers BMS500BT-20, BMS500BT-100 and BMS500BT-300

Pub. No. MAN0016664 **Rev.** A.0 (30)

 **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 use

The eBioscience™ Annexin V-Biotin Apoptosis Detection Kit can be used to detect phosphatidylserine on the outer leaflet of the cell membrane using flow cytometry.

Summary

Annexins are a family of calcium-dependent phospholipid-binding proteins. They are abundant in eukaryotic organisms belonging to a family of ubiquitous cytoplasmic proteins involved in signal transduction. All annexins have been shown to have a putative binding site for protein kinase C (PKC) but only annexin V would possess a potential pseudo-substrate site. Thus annexin V seems to modulate the activity of some PKCs on their substrates.

Annexin V was found to play a major role in matrix vesicle-initiated cartilage calcification as a collage-regulated calcium channel. Annexin V binds to procoagulant phospholipids (vascular anticoagulant alpha) with high affinity.

Annexin V's preferential binding partner is phosphatidylserine (PS). PS is predominantly located in membrane leaflets, which face the cytosol. However, recent findings show that each cell type has the molecular machinery to expose PS at its cell surface. This machinery is activated during the execution of apoptosis. When PS is exposed at the cell surface it exhibits procoagulant and proinflammatory activities. Annexin V will bind to the PS-exposing apoptotic cell and can inhibit the procoagulant and proinflammatory activities of the dying cell.

For literature update refer to our website.

Principles of the test

Annexin V exhibits antiphospholipase activity and binds to phosphatidylserine. Biotin labelling allows simple direct detection by FACS analysis. Counterstaining by propidium iodide allows the discrimination of apoptotic cells.

Reagents provided

	BMS500BT/20 for 20 tests	BMS500BT/100 for 100 tests	BMS500BT/300 for 300 tests
rh Annexin V-Biotin	1 vial (100 µL)	1 vial (500 µL)	1 vial (1.5 mL)
Binding Buffer (4x)	1 bottle (50 mL)	1 bottle (50 mL)	2 bottles (50 mL)
Propidium Iodide (20 µg/mL)	1 vial (1.8 mL)	1 vial (1.8 mL)	2 vials (1.8 mL)

Storage and stability

Store kit reagents between 2° and 8°C. Immediately after use remaining reagents should be returned to cold storage (2–8°C). After opening aliquot Annexin V-Biotin and store at –20°C.

Expiry of the kit and reagents is stated on labels. Expiry of the kit components can be guaranteed only if the components are stored

properly, and if, in case of repeated use of one component, this reagent is not contaminated by the first handling.

Materials required but not provided

- 5 mL and 10 mL graduated pipettes
- 5 µL to 1000 µL adjustable single-channel micropipettes with disposable tips
- Beakers, flasks, and cylinders necessary for preparation of reagents
- Glass-distilled or deionized water
- Bench top centrifuge
- Flow Cytometer
- PBS (for 1 liter: 8.00 g NaCl, 0.20 g, 2.85 g Na₂HPO₄ × 12 H₂O, 0.20 g KH₂PO₄)
- Fluorescence-labeled Streptavidin

Precautions for use

- All reagents should be considered as potentially hazardous. We therefore recommend that this product is handled only by those persons who have been trained in laboratory techniques and that it is used in accordance with the principles of good laboratory practice. Wear suitable protective clothing such as laboratory overalls, safety glasses and gloves. Care should be taken to avoid contact with skin or eyes. In the case of contact with skin or eyes wash immediately with water. See material safety data sheet(s) and/or safety statement(s) for specific advice.
- Reagents are intended for research use only and are not for use in diagnostic or therapeutic procedures.
- Do not mix or substitute reagents with those from other lots or other sources.
- Do not use kit reagents beyond expiration date on label.
- Do not expose kit reagents to strong light during storage or incubation.
- Do not pipette by mouth.
- Do not eat or smoke in areas where kit reagents or samples are handled.
- Avoid contact of skin or mucous membranes with kit reagents or samples.
- Rubber or disposable latex gloves should be worn while handling kit reagents or samples.
- Avoid splashing or generation of aerosols.
- To avoid microbial contamination or cross-contamination of reagents or samples that may invalidate the test, use disposable pipette tips and/or pipettes.
- Glass-distilled water or deionized water must be used for reagent preparation.
- Decontaminate and dispose samples and all potentially contaminated materials as if they could contain infectious agents. The preferred method of decontamination is autoclaving for a minimum of 1 hour at 121.5°C.
- Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 1.0% sodium hypochlorite. Allow 30 minutes for effective decontamination. Liquid waste containing acid must be neutralized prior to the addition of sodium hypochlorite.

Preparation of reagents

Dilute Binding Buffer (4x) 1:4 in distilled water (50 mL binding buffer and 150 mL distilled water).

Test protocol

1. Wash cells in PBS by gentle shaking or pipetting up and down.
2. Resuspend cells in 200 μ L Binding Buffer (1X); cell density should be $2-5 \times 10^5$ /mL.
3. Add 5 μ L Annexin V-Biotin to 195 μ L cell suspension.
4. Mix and incubate for 15 minutes at room temperature.
5. Wash cells 2X and resuspend in Binding Buffer (1X).
6. Add fluorescence-labeled Streptavidin.
7. Mix and incubate for 30 minutes in the dark, at room temperature.
8. Wash cells in 200 μ L Binding Buffer (1X) and resuspend in 190 μ L Binding buffer (1X).
9. Add 10 μ L Propidium Iodide (20 μ g/mL)
10. Perform FACS analysis.

Limitations

Bacterial or fungal contamination of either screen samples or reagents or cross-contamination between reagents may cause erroneous results.

Disposable pipette tips, flasks, or glassware are preferred, reusable glassware must be washed and thoroughly rinsed of all detergent before use.

Customer and technical support

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- Worldwide contact telephone numbers
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 - User guides, manuals, and protocols
 - Certificates of Analysis
 - Safety Data Sheets (SDSs; also known as MSDSs)

Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

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's address: Bender MedSystems GmbH | Campus Vienna Biocenter 2 | 1030 Vienna, Austria

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