PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

Annexin V

FITC	RUO	REF	IQP-120F	100 tests
R-PE	RUO	REF	IQP-120R	100 tests
APC	RUO	REF	IQP-120A	100 tests

RUO *For Research Use Only*

Description

Annexin V

During the early stages of apoptosis, phosphatidylserine (PS) becomes exposed on the outside of the cell membrane. This can be specifically detected by PS binding proteins, such as, Annexin V.

Summary

Annexin V possesses anticoagulant properties and is proven to be a useful tool in detecting apoptotic cells since it preferentially binds to negatively charged phospholipids like PS in the presence of calcium (Ca2+) and shows minimal binding to phosphatidylcholine and sphingomyeline. Changes in PS asymmetry, analyzed by measuring Annexin V binding to the cell membrane, can be detected prior to morphological changes associated with apoptosis and loss of membrane integrity.

Background

Apoptosis, or programmed cell death (PCD), is a genetically encoded cell elimination program which ensures an equilibrium between cell proliferation and cell death and by which damaged or unwanted cells are eliminated. Apoptosis is a normal physiological process and without continuous signaling by growth factors, hormones or cytokines all cells undergo PCD. Aberrations in the mechanism of apoptosis are involved in the occurrence of congenital defects, malignancies, autoimmune diseases, immune deficiency syndromes and in degenerative conditions. Apoptosis involves physiological cell elimination in contrast to necrosis, pathological cell death, induced by physical or chemical injury. PCD plays an integral part in tissue repair and homeostasis of the organism. During necrosis the cell membrane loses its selective permeability and ion-pumping capacity. Necrosis occurs in whole fields of damaged cells, where the leaked cellular debris elicits an inflammatory reaction in the surrounding viable tissues. During apoptosis the integrity of the cell membrane and the mitochondria remains initially intact, the cytoplasm condenses and the nucleus breaks up into DNA fragments. The cell adopts a convoluted outline and subsequently breaks up into small vesicles, known as apoptotic bodies, which enclose the cell remains and end up in the extracellular space. The cell remnants are phagocytosed by nearby cells and eliminated without eliciting any inflammatory reaction.
The process of apoptotic cell death takes only from a few minutes to some hours. It involves single scattered cells, the remnants of which are quickly eliminated by phagocytosis, which means that the measurement of apoptosis in a sensitive, specific and quantitative manner is not simple. One method measures apoptosis in cell suspensions by loss of membrane asymmetry using flow cytometry3. During the early stages of apoptosis, PS becomes exposed on the outside of the cell membrane. This can be specifically detected by PS binding proteins, such as, Annexin V. Necrotic cells are permeable for PI, which intercalates in nuclear DNA and is visible as red fluorescence.

Applications

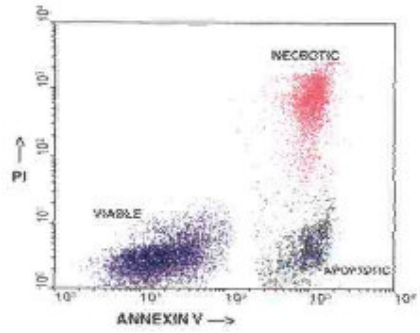
FITC conjugated Annexin V makes it possible to identify and quantitate apoptotic cells on a single-cell basis by flow cytometry. Staining cells simultaneously with Annexin V (FITC) and the non-vital dye propidium iodide allows the discrimination of intact cells (FITC-PI-), early apoptotic (FITC+PI-) and late apoptotic or necrotic cells (FITC+PI+).

Usage

All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/10^6 leukocytes. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative data

Staining with Annexin V FITC is illustrated by flowcytometry analysis of apoptotic lymphocytes. Direct staining was performed using 10 µl Annexin V FITC and 100 µl isolated lymphocytes (according to Prosphatidyl Serine detection kit protocol).



Combining PI (propidium iodide) and PS binding proteins results in a fast and reliable tool for detection and quantitation of apoptosis in a single cell basis. The figure shows a dot plot of Annexin V FITC and PI analysis of HSB-2 cells 4 hours after irradiation (8 Gray) (Haanen and Vermes 1998).

Limitations

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
3. Reagents can be used in different combinations, therefore laboratories need to become familiar performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples.
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Reagents and materials required but not supplied

1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. PBS (phosphate-buffered saline)

Procedure

Isolation of WBCs from whole blood

1. Dilute the calcium buffer* 20 times in demineralized water and keep at 4 °C.
2. Isolate WBCs from a whole blood by a standard Isopaque procedure.
3. Add 5-10 ml of diluted-cold calcium buffer to the WBCs.
4. Mix by subtle vortexing, spin down 2 minutes at 1000 g and discard supernatant.
5. Repeated washing is possible but not necessary.
6. Resuspend the cells in suitable amount of buffer (1 ml) and estimate the cell concentration by cell counter.
7. Readjust to the concentration mentioned in protocols below.

Protocol 1 - Detection of Phosphatidyl Serine after incubation with apoptosis inducing agents

8. Prepare a 100 µM dexamethasone solution (Sigma D1756) to induce apoptosis (3.4 mg of dexamethasone in 1 ml methanol).
9. Prepare a cell suspension of $1.0x10^6$ cells/ml.
10. Add 1 mL of cell suspension per well (24-well plate).
11. Add 10 µL dexamethasone solution to the cell suspension.
12. Incubate for 1 or 2 day(s) at 37 °C in a CO_2 incubator.
13. Dilute the calcium buffer* 20 times in demineralized water and keep at 4 °C.
14. Remove the cells from the well (24 well plate) and add 2 volumes of calcium buffer.
15. Mix gently and centrifuge 3 minutes at 400 g.
16. Repeat the washing.

17. Re-adjust the cell concentration to $1.0x10^6$ cells/ml.
18. Add 10 µl Annexin V FITC to 100 µl cell suspension and incubate for 20 minutes at 4 °C.
19. Wash the cells again with calcium buffer (2 volumes; 3 minutes at 400 g).
20. Repeat the washing.
21. Add 10 µl propidium iodide and incubate for at least 10 minutes at 4 °C.
22. Keep the cells at 4 °C until ready to analyze by flow cytometer.

Protocol 2 - Detection of Phosphatidyl Serine in unstimulated cells (or ex vivo)

8. Dilute the calcium buffer* 20 times in demineralized water and keep at 4 °C.
9. Add 2 volumes of calcium buffer to the cells.
10. Mix gently and centrifuge 3 minutes at 400 g.
11. Re-adjust the cell concentration to $1.0x10^6$ cells/ml in calcium buffer.
12. Add 10 µl Annexin V FITC (PE or APC) to 100 µl cell suspension and incubate for 20 minutes on ice, in the dark.
13. Wash the cells with calcium buffer (2 volumes; 3 minutes at 400 g).
14. Add 10 µl Propidium iodide and incubate for at least 10 minutes on ice.
15. Keep the cells at 4 °C until ready to analyze by flow cytometer.

Protocol 3 - Dual staining: Phosphatidyl Serine plus membrane antigen

1. Follow the protocol as described for stimulated or unstimulated cells.
2. Add the labeled monoclonal antibody to the membrane antigen at the same time as the Annexin V.
3. Add 10 µl Annexin V FITC to 100 µl cell suspension and incubate for 20 minutes at 4 °C.
4. Wash the cells again with calcium buffer (2 volumes; 3 minutes at 400 g).
5. Repeat the washing.
6. Add 10 µl propidium iodide and incubate for at least 10 minutes at 4 °C.
7. Keep the cells at 4°C until ready to analyze by flow cytometer.

* Calcium buffer for labeling cells with Annexin V

Preparation of a 20x stock solution.
Dissolve in 50 ml of demineralized water the following components:

- Hepes (2.60 g)
- NaCl (8.18 g)
- $CaCl_2$ (0.28 g)

Before use dilute 1.0 ml of calcium buffer in 20 ml of demineralized water.

Notes

1. The Phosphatidyl Serine detection assay detects apoptotic cells well before DNA-based assays, since externalization of PS occurs prior to the nuclear changes associated with apoptosis.
2. Normally, PI uptake is a sign of death by necrosis. In vivo, apoptotic cells are removed by phagocytic cells before losing membrane integrity and would normally not show PI uptake.
3. Annexin V is a 35kDa single chain protein that is dependent on Ca2+ for its binding to Phosphatidyl Serine.
4. It is recommended that the Phosphatidyl Serine detection assay should be performed on ice in order to prevent further progression from viable cells to apoptotic cells or necrosis.
5. The Phosphatidyl Serine detection assay has been found to detect apoptosis in a wide variety of cell suspensions, including peripheral blood lymphocytes (3), thymocytes (4) and germinal center B cells (5).

Handling and Storage

Antibodies are supplied either as 100 tests per vial (1 ml) for singles or 50 tests per vial (1 ml) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN_3). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly.

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product.

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only.

References

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2. Jacobson, M.D., 1997. Programmed cell death in animal development. Cell 88: 347-354
3. Vermes et al. 1995. A novel assay for apoptosis: Flow cytometric detection of phosphatidylserine expression on early apoptotic cells using fluorescein labeled Annexin V. J. Immunol. Methods. 184. 39-51
4. Martin, S.J., et al., 1995. Early redistribution of plasma membrane phosphatidylserine is a general feature of apoptosis regardless of the initiating stimulus. Inhibition by over expression of Bcl-2 and Abl. J.Exp. Med. 182, 1545 – 1557
5. Koopman et al. 1994. Annexin V for flow cytometric detection of phosphatidylserine expression on B cells undergoing apoptosis. Blood. 84, 1415-1420Vermes,I., Haanen,C., Reutelingsperger, CPM., Apoptosis – the genetically controlled physiological cell death: bilchemistry and measurement. 1997. 22: 43 –50

Explanation of used symbols

	Consult instructions for use
REF	Catalogue number
	Sufficient for
IVD	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
RUO	For Research Use Only
LOT	Batch code
	Use by yyyy-mm-dd
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	Conformité Européenne (European Conformity)

		Label - tandem	**Ex -max (nm)**	**Em -max (nm)**
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
C	CyQ	PE-Cy5.18	488, 532	667
A	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695

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