PRODUCT INFORMATION SHEET

Propidium Iodide for (apoptotic) cell death analysis

Propidium Iodide

REF IQP-121 100 tests

RUO ***For Research Use Only***

Propidium Iodide

Propidium Iodide (PI) is a fluorescent DNA-binding dye. It freely penetrates cell membranes of dead or dying cells, but is excluded from viable cells. These properties favor the use of PI for evaluation of (apoptotic) cell death or for cell cycle analysis. When excited by a 488 nm laser, the fluorescence emission maximum for DNA-bound PI is about 615–620 nm. Usually, PI fluorescence is detected in the FL2 channel of flow cytometers.

Background

Determination of cell viability is an important parameter to monitor the response to cytotoxic drugs or other environmental factors.
The process of cell death can be separated in apoptotic or necrotic cell death. 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.
Flow cytometry provides a rapid and reliable method to quantify cell death in a cell suspension. 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

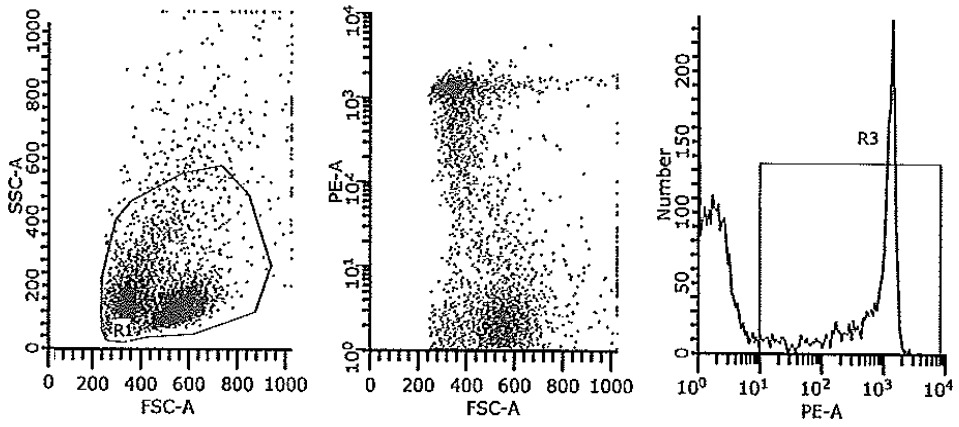
Propidium Iodide makes it possible to identify and quantitate dead and necrotic cells on a single-cell basis by flow cytometry. Staining cells simultaneously with the apoptosis marker Annexin V (FITC) and propidium iodide allows the discrimination of intact cells (FITC-PI-), early apoptotic (FITC+PI-) and late apoptotic or necrotic cells (FITC+PI+). If you are interested in this application, please consult the package insert of IQP-116F/R.

Usage

PI is 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 Propidium Iodide is illustrated by flow cytometry analysis of apoptotic lymphocytes. Direct staining was performed using 10 µl Propidium Iodide and 100 µl isolated lymphocytes.



Limitations
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. IQ Lyse - erythrocyte lysing solution (IQP-199)
7. IQ Starfiqs – fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Immunofluorescence staining and lysing protocol

Flow cytometry method for use with PI

1. Add 100 µl of EDTA-treated blood (i.e. approx. 10^6 leukocytes) to a 5 ml reagent tube. The content of one tube is sufficient to perform one test.
2. Add to each tube 10 µl of PI. Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for at least 10 minutes at room temperature in the dark.
4. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
5. Remove the supernatant and resuspend the cells in 200 µl of PBS.*
6. Analyze by flow cytometry within four hours (alternatively, the cells may be fixed by 0.05% of formaline in buffered saline for analysis the next day. Some antigens are readily destroyed upon fixation and this should be taken into account when using this alternative).

** PBS: Phosphate Buffered Saline, pH 7.2*

Handling and Storage
Propidum Iodide is supplied as 100 tests per vial (1 ml). It is 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. Propidium Iodide 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 propidium iodide is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet.

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

References

1. Van der Toorn M, Slebos DJ, De Bruin HG, Leuvenink HG, Bakker SLJ, Gans ROB, Koëter GH, Van Oosterhout AJM and Kauffman HF Cigarette smoke-induced blockade of the mitochondrial respiratory chain switches lung epithelial cell apoptosis into necrosis. *Am J Physiol Lung Cell Mol Physiol* ***292****: L1211–L1218, 2007.*
2. Elsayed SM and Elsayed GM Phenotype of apoptotic lymphocytes in children with Down syndrome. *Immunity & Ageing* **6**:2 2009
3. Lapter S, Ben-David H, Sharabi A, Zinger H, Telerman A, Gordin M, Leng L, Bucala R, Shachar I, Mozes E. A role for the B-cell CD74/macrophage migration inhibitory factor pathway in the immunomodulation of systemic lupus erythematosus by a therapeutic tolerogenic peptide. *Immunology* ***132****(1):87-95, 2011.*

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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