

PRODUCT INFORMATION SHEET

Merocyanine 540 for (apoptotic) cell death analysis

Merocyanine 540

PURE RUO REF IQP-198 ▼ 100 tests

RUO **For Research Use Only**



Description

Summary Merocyanine 540 is a fluorescent lipophilic probe which binds preferentially to membranes with loosely packed lipids and as such stains apoptotic cells more brightly than normal cells. This change is due to an increase in fluorescence, which occurs with the loss of membrane symmetry. Lymphocytes undergoing apoptosis loosen the packing of lipids on their surface. Changes in merocyanine 540 fluorescence have been shown to generally correlate with the translation of membrane phosphatidyl serine from the inner to the outer leaflet of the plasma membrane ¹.

Applications Merocyanine 540 stains intact cells so that cells programmed to die but still undisrupted can be sorted from non-apoptotic cells on the basis of their increased Merocyanine 540 staining. In general there is a close correlation between Annexin V and Merocyanine 540 in staining pattern. However small differences in percentage of stained cells may be seen in some cell types². The advantage of using Merocyanine 540 is that it is a simple one step staining procedure which can be combined with an antibody for a cell membrane antigen allowing phenotypic analysis of the apoptotic cells. See figure 1 (a) below.

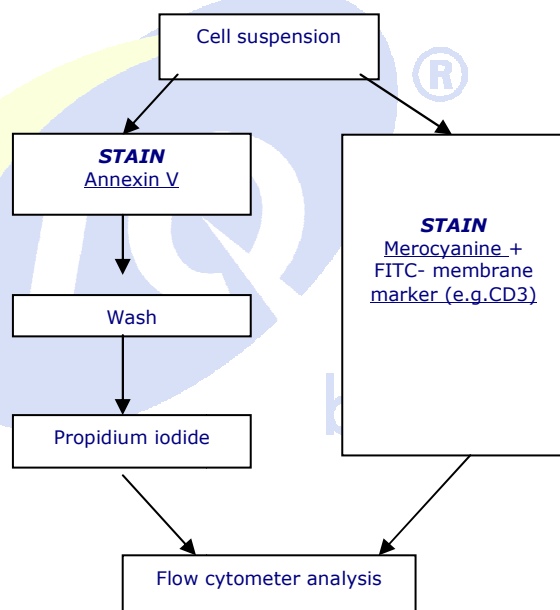


Fig. 1(a) Flow diagram of the steps used to stain apoptotic cells with Merocyanine 540 or Annexin V and PI.

Usage

- Instructions for using Merocyanine 540
- Add 10 µl Merocyanine 540 (IQP-198) to 100 µl cell suspension.
 - Incubate for 15 minutes.
 - Analyze by flow cytometer.
 - Merocyanine 540: excitation 488 nm/emission 560; Annexin V excitation 488 nm/emission 512)

Representative Data

Peripheral blood lymphocytes are incubated overnight with dexamethasone to induce apoptosis. The cells are then stained with Annexin V FITC and Merocyanine 540. Figure 1b shows that most but not all Merocyanine 540 positive cells also stain positively with Annexin V. This may mean that Merocyanine binding occurs before that of Annexin V or that some apoptotic cells are not stained by Annexin V². However in general there is a close correlation between Annexin V and Merocyanine 540 in staining pattern.

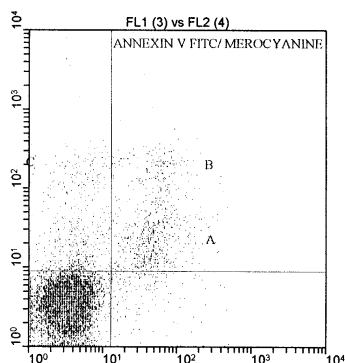


Fig. 1(b) Cytogram showing Annexin V FITC Merocyanine 540 labeling of peripheral blood lymphocytes.

Limitations

1. 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.
2. 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.
3. 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)

* PBS: Phosphate Buffered Saline, pH 7.2



Handling and Storage

Merocyanine 540 is supplied either as 100 tests per vial. It is supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 7.3, 0.2% BSA, 0.09% sodiumazide (NaN₃). Store the vials at 2-8 °C. Merocyanine 540 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 Merocyanine 540 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

1. Castedo, M., et al., 1996 J. Immunol. 157. 512-521
2. Frey, T., 1997. Cytometry, 28. 253-263

Explanation of used symbols

	Consult instructions for use
	Catalogue number
	Sufficient for
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
	For Research Use Only
	Batch code
	Use by yyyy-mm-dd
	Manufacturer

		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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