PRODUCT INFORMATION SHEET

IQ Starfiqs™ - Intracellular staining by flow cytometry

IQ Starfiqs™

Intracellular staining of cytokines and other intracellular antigens

RUO REF IQP-200 50 tests

RUO ***For Research Use Only***

Description

Summary

IQ Starfiqs™ is composed of two reagents for the fixation and permeabilization of prepared blood leukocytes. IQ Starfiqs™ is provided as a ready-to-use product, to minimize hand-on time and the easy handling of samples. IQ Starfiqs™ is intended for the optimum flow cytometric analysis of intracellular antigens.

Fixation and permeabilization with IQ Starfiqs™ enables the detection of intracellular cytokines, intracellular antigens such as CyCD3, CyCD22, TdT and MPO, and has also been used successfully to fixate and permeabilize cells in the TUNEL assay for detection of apoptosis. In addition, IQ Starfiqs™ allows the simultaneous detection of cell surface antigens.

Note - For optimal intracellular immuno-staining and lysing of erythrocytes in case of whole blood protocols, IQ Starfiqs™ should be used following the procedures described. The protocol will depend on the type of antigen and whether whole blood or isolated lymphocytes are being used. It is important to use both reagents and not to mix with other products.

Applications

Detection of intracellular antigens in whole blood, bone marrow suspensions or isolated leukocytes.

Usage

This information sheet contains protocols for the detection of:

A - Intracellular cytokines
- 1 - whole blood method
- 2 - isolated leukocytes

B - Other intracellular antigens (such as CyCD3, CyCD22, TdT and MPO)
- 3 - whole blood method
- 4 - isolated leukocytes

Both protocols are suitable for dual staining; simultaneous detection of intracellular antigens and cell surface antigens such as CD3, CD4 and CD8.

Protocols for the immuno-fluorescence staining of intracellular antigens

- A - Detection of intracellular cytokines

- 1 - whole blood protocol

1 Dilution of whole blood
   a. Collect 1-3 ml venous blood into a heparinized treated tube by aseptic venipuncture
   b. Dilute the blood sample 1:10 with RPMI 1640 and mix well
   c. Transfer 1 ml of the ceIl suspension into a 24 well culture plate

Note *5 ml of cell suspension is sufficient for intracellular detection of five different cytokines*

2. Stimulation of cells, if required, according to published protocols (1)
   a. After stimulation, collect the cells and transfer the cell suspension (5 ml) to a centrifuge tube
   b. Centrifuge at 200 g for 10 minutes and remove supernatant

3. Fixation of cells
   a. Add 500 µl IQ Starfiqs™ fixation reagent (Reagent F)
   b. Incubate 10 minutes at room temperature
   c. Add 9 ml HBSS (Hanks Buffered Saline Solution)
   d. Centrifuge at 200 g for 10 minutes and remove the supernatant
   e. Resuspend the cells in 1 ml HBSS
   f. Store the cells overnight at 4 °C

4 Staining of cell surface antigens
   a. After fixation, add 5 ml of HBSS to the cell suspension and centrifuge at 200 g for 10 minutes
   b. Remove the supernatant and resuspend the cells in 500 µl of HBSS. This cell suspension is sufficient for five separate experiments of 100 µl per experiment
   c. Place 20 µl of fluorochrome-conjugated monoclonal antibody (specific for the cell surface antigen) in a 5 ml tube
   d. Add 100 µl of cell suspension to the tube and mix well by vortexing, and incubate for 20 minutes at room temperature in the dark
   e. Add 4 ml HBSS. Centrifuge at 200g for 10 minutes. Remove supernatant

5 Permeabilization and intracellular staining
   a. Add 10 µl of conjugated monoclonal antibody directed against the intracellular antigens in a reagent tube
   b. Add 200 µl IQ Starfiqs™ permeabilization solution (Reagent P)
   c. Incubate for 20 minutes at 4 °C in the dark
   d. Add 4 ml HBSS
   e. Centrifuge at 1200 rpm for 10 minutes
   f. Remove the supernatant and resuspend the cells in 150 µl of PBS (phosphate buffered saline)

6. Analysis by flow cytometry
   a. Appropriate controls may include unlabeled monoclonal antibody (blocking) or isotype controls

- 2 - isolated leukocytes

1. Isolation of PMNs by density gradient centrifugation (Ficoll-Paque)
2. Resuspend cells to 1 x 10^6 cells per ml
3. Add 100 µl cell suspension to a reagent tube
4. Add 10 µl of antibody-conjugate directed against a cell surface antigen
5 Incubate for 15 minutes at room temperature in the dark
6. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg
7. Remove the supernatant
8. Add 100 µl IQ Starfiqs™ fixation reagent (Reagent F)
9. Incubate for 15 minutes at room temperature
10. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
11. Remove the supernatant and add 10 µl of IQ Products antibody-conjugate directed against an intracellular antigen
12. Add 100 µl of IQ Starfiqs™ permeabilization reagent (Reagent P)
13. Incubate for 15 minutes at room temperature
14. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
15. Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline
16. Analysis by flow cytometry

- B - Intracellular antigens such and CyCD3, CyCD22, TdT and MPO

- 1 - whole blood protocol

1. Add antibody conjugate to a reagent tube: 10 µl of antibody-conjugate directed against a cell surface antigen
2. Add 100 µl of EDTA- or Heparin-treated whole blood and mix well
3. Incubate for 15 minutes at room temperature in the dark
4. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
5. Remove the supernatant
6. Add 100 µl IQ Starfiqs™ fixation reagent (Reagent F)
7. Incubate for 15 minutes at room temperature
8. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
9. Remove the supernatant and add 10 µl of IQ Products antibody-conjugate directed against an intracellular antigen
10. Add 100 µl of IQ Starfiqs™ permeabilization reagent (Reagent P)
11. Incubate for 15 minutes at room temperature
12. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
13. Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline
14. Analysis by flow cytometry

- 2 - isolated leukocytes

1. Isolation of PMNs by density gradient centrifugation (Ficoll-Paque)
2. Resuspend cells to 1 x 10^6 cells per ml
3. Add 100 µl cell suspension to a reagent tube
4. Add 10 µl of antibody-conjugate directed against a cell surface antigen
5 Incubate for 15 minutes at room temperature in the dark
6. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg
7. Remove the supernatant
8. Add 100 µl IQ Starfiqs™ fixation reagent (Reagent F)
9. Incubate for 15 minutes at room temperature
10. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
11. Remove the supernatant and add 10 µl of IQ Products antibody-conjugate directed against an intracellular antigen
12. Add 100 µl of IQ Starfiqs™ permeabilization reagent (Reagent P)
13. Incubate for 15 minutes at room temperature
14. Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300 g
15. Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline
16. Analysis by flow cytometry

Handling and Storage

Store the vials at 2 to 8 °C. 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

1. Jung et al., 1993. Detection of intracellular cytokines by flow cytometry. J.Immunol. Meth. 159. 197-207

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

IQ Products BV
Rozenburglaan 13a
9727 DL Groningen, The Netherlands

+31 (0)50 57 57 000
+31 (0)50 57 57 002
Technical marketing@iqproducts.nl
Orders orders@iqproducts.nl
www.iqproducts.nl