



Protocols

IQ Starfiqs™ Intracellular Staining of Cytokines and other Intracellular Antigens

Applications

Whole blood or bone marrow suspension
Isolated leukocytes

Protocols for the detection of

A - Intracellular cytokines

- (a) Whole blood method
- (b) Isolated leukocytes

B - Other intracellular antigens (such as CyCD3, CyCD22, TdT and MPO)

- (a) Whole blood method
- (b) Isolated leukocytes

Suitable for dual staining

Simultaneous detection of intracellular antigens and cell surface antigens (such as CD3, CD4, CD8).

IQ Starfiqs™ is a fixation and permeabilization solution intended for preparation of blood leukocytes for flow cytometry analysis of intracellular antigens. **IQ Starfiqs™** is a **ready to use** product, and is suitable for use with whole blood or isolated leukocytes. It is composed of two reagents used sequentially and they have been developed to ensure optimum performance in flow cytometry.

Both reagents should be stored at 4–8 °C.

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 fix and permeabilize cells in the TUNEL assay for apoptosis. In addition, **IQ Starfiqs™** allows the simultaneous detection of cell surface antigens.

For optimal intracellular immuno-staining and lysing of erythrocytes in the 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. **IQ Starfiqs™** is provided as a ready to use product, to minimize hands on time and the easy handling of samples.

Protocol for immuno-fluorescence staining of intracellular antigens

A. Detection of Intracellular Cytokines:

(a) Whole blood protocol

1. Dilution of whole blood

- Collect 1-3 ml venous blood into a heparinized treated tube by aseptic venipuncture.
- Dilute the blood sample 1:10 with RPMI 1640 and mix well.
- Transfer 1 ml of the cell 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. (Jung et al 1993^{*}).

- After stimulation, collect the cells and transfer the cell suspension (5 ml) to a centrifuge tube.
- Centrifuge at 200 g for 10 minutes and remove supernatant.

3. Fixation of cells

- Add 500 μ l **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate 10 minutes at room temperature.
- Add 9 ml HBSS (Hanks buffered saline solution)
- Centrifuge at 200 g for 10 minutes and remove the supernatant.
- Resuspend the cells in 1ml HBSS.
- Store the cells overnight at 4°C.

4. Staining of cell surface antigens

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

5. Permeabilization and intracellular staining

- Add 10 μ l of R-PE or FITC conjugated monoclonal antibody directed against the intracellular antigens in a reagent tube.
- Add 200 μ l **IQ Starfiqs™** permeabilization solution (Reagent P).
- Incubate for 20 minutes at 4° C in the dark.
- Add 4 ml HBSS.
- Centrifuge at 1200 rpm for 10 minutes.
- Remove the supernatant and resuspend the cells in 150 μ l of PBS (phosphate buffered saline).

6. Analysis by flow cytometry

- Appropriate controls may include unlabeled monoclonal antibody (blocking) or isotype controls.

(b) Isolated leukocytes

1. Isolation of PMNs by density gradient centrifugation (Ficoll-Paque)
2. Resuspend cells to 1×10^6 cells per ml.
3. Stimulate cells, if required, according to published protocols. (Jung et al 1993^{*}).
4. After stimulation, transfer the cell suspension to a centrifuge tube .
5. Centrifuge at 1200 rpm for 10 minutes and remove supernatant.
6. Fixation of cells.
 - Add 100 μ l **IQ Starfiqs** fixation reagent (Reagent F) to the cells: maximal 3×10^6 cells per tube.
 - Incubate for 10 min at room temperature.Complete the procedure as described above for whole blood: see (3) Fixation of cells.

B. Intracellular antigens: such as CyCD3, CyCD3, CyCD22, TdT, MPO

(a) Whole blood method.

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.

(b) Isolated leukocytes

1. Isolation of PMNs by density gradient centrifugation (Ficoll-Paque)
2. Resuspend cells to 1×10^6 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. Proceed as for the whole blood method above: see step 7.