

## PRODUCT INFORMATION SHEET

### Monoclonal antibodies detecting human antigens

**CD3** FITC    **CD8** R-PE    REF    IQP-254FR    IVD    50 tests

IVD        *In Vitro Diagnostic medical device*



#### Description

**CD3**    Clone    **UCHT1**    Isotype    **murine IgG1**  
 For detailed description of this particular single reagent, please refer to IQP-519, CD3 (UCHT1)

**CD8**    Clone    **MCD8**    Isotype    **murine IgG1**  
 For detailed description of this particular single reagent, please refer to IQP-104, CD8 (MCD8)

**Intended use**    CD3/CD8 dual combination, IQP-254FR, is a direct immunofluorescence reagent used for the detection of mature human T suppressor/cytotoxic lymphocytes (CD3+/CD8+) in whole blood (either lysed or non-lysed) using flow cytometry.

**Summary**    Human lymphocytes are, based on biological function and cell-surface antigen expression, divided in three populations: T, and B lymphocytes, and natural killer (NK) lymphocytes. T lymphocytes participate in antigen-specific cell-mediated immunity and regulate the secretion of immunoglobulin by B lymphocytes. T lymphocytes may also be classified based on their functional properties as helper/inducer and suppressor/cytotoxic.

**Applications**    Suppressor/cytotoxic lymphocytes are a subset of T lymphocytes (CD3+) that are CD8+. The amount of T suppressor/cytotoxic cells (CD3+ CD8+) detected may be used to characterize and monitor some forms of immunodeficiency and autoimmune diseases.

The amount of T suppressor/cytotoxic cells may change from the normal values in some autoimmune diseases, certain immune reactions like acute graft-versus-host disease (GVHD) and transplant rejection. The relative amount of CD8+ cells is elevated in many patients with either congenital or acquired immune deficiencies, such as severe combined immunodeficiency (SCID) and acquired immune deficiency syndrome (AIDS). The CD8+ lymphocyte population may be lowered in active systemic lupus erythematosus (SLE) but can also be increased in SLE patients undergoing steroid therapy.

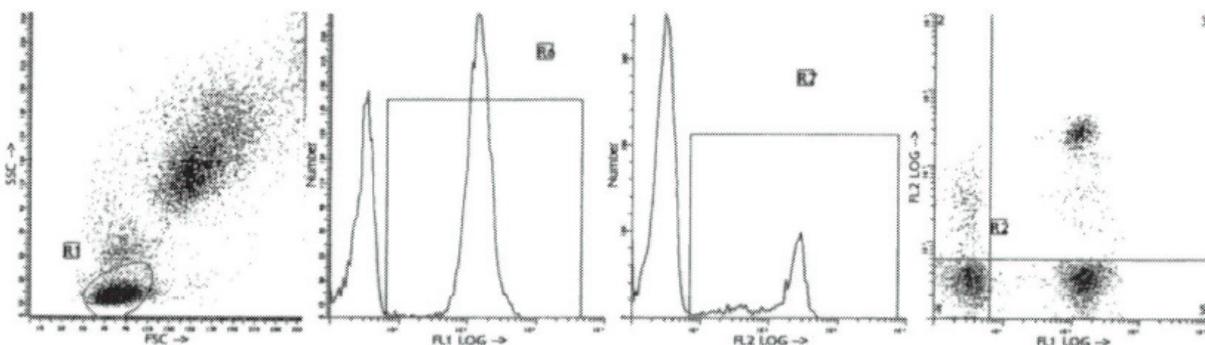
Using IQP-254FR, T suppressor/cytotoxic cells are detected separately from interfering CD3-CD8+ NK cells.

Note: Not all the application mentioned are performed using IQ Products reagents.

Note: The Centers for Disease Control (CDC) recommends using a dual color reagent combination containing CD3 and CD8 antibodies for determining the percentage of CD8+ T lymphocytes, and a dual color reagent combination containing CD3 and CD4 antibodies for determining the percentage of CD4+ T lymphocytes in human immunodeficiency virus (HIV)-infected patients.

**Usage**    All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µL/10<sup>6</sup> leukocytes for singles and 20 µL/10<sup>6</sup> leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

#### Representative Data



## Reproducibility

Monoclonal antibodies from IQ Products were tested by flow cytometry using a 'lyse-wash' method on whole blood from healthy donors. Obtained data support the premise that these reagents are equivalent in their reactivity with peripheral blood lymphocytes. Values are expressed in terms of % of the total lymphocyte count (see table).

| Reagent  | n  | Mean % positive | S.D. | % CV  | Product code |
|----------|----|-----------------|------|-------|--------------|
| CD3 FITC | 10 | 66,93           | 6,90 | 10,31 | IQP-254FR    |
| CD8 R-PE | 10 | 20,31           | 3,44 | 16,95 | IQP-254FR    |

## 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. IQ Lyse - erythrocyte lysing solution (IQP-199)
7. IQ Starfiqs - fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% Heparin
10. 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 dual and triple combinations

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.  
**For combinations with anti-kappa and/or anti-lambda Ig see application note below.**
2. Add to each tube 20 µl of labeled monoclonal antibody combination.\*
3. Vortex the tube to ensure thorough mixing of antibody and cells.
4. Incubate the tube for 15 minutes at room temperature in the dark.
5. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately.
6. Incubate for 10 minutes at room temperature in the dark.
7. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
8. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
9. Remove the supernatant and resuspend the cells in 200 µl of PBS.\*\*
10. 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).

\* Appropriate mouse Ig isotype control samples should always be included in any labeling study

\*\* PBS: Phosphate Buffered Saline, pH 7.2

### **Application note for anti-kappa and/or anti-lambda Ig combinations**

Add 2 ml of PBS containing 0.001% (v/v) Heparin (**prewarmed to 37 °C**) to the cell suspension  
Vortex, centrifuge (2 min at 300x g) and discard the supernatant  
Repeat this step twice  
Resuspend the pelleted blood cells in 100 µl PBS containing 0.001% (v/v) Heparin



### 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<sub>3</sub>). 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.

### Explanation of used symbols

|  |                                                     |
|--|-----------------------------------------------------|
|  | Consult instructions for use                        |
|  | Catalogue number                                    |
|  | Sufficient for                                      |
|  | In Vitro Diagnostic medical device                  |
|  | 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                                        |
|  | Authorized Representative in the European Community |
|  | Conformité Européenne (European Conformity)         |

|     |             | <b>Label - tandem</b> | <b>Ex -max (nm)</b> | <b>Em -max (nm)</b> |
|-----|-------------|-----------------------|---------------------|---------------------|
| 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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