# PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

## CD79a

R-PE RUO REF IQP-122R 100 tests

RUO ***For Research Use Only***

### Description

**Clone** HM57

**Isotype** murine IgG1

**Specificity** CD79a (HM57) recognizes a 45 kD human B cell antigen, also known as MB-1.

**Antigen distribution**

CD79a is expressed on B lineage cells and forms a complex with CD79b and is part of the B Cell Receptor (BCR) complex. It is expressed by B lymphocytes during differentiation from early pre-B cell stage through to plasma cells.

The antibody has (cross-)reactivity with: mouse, dog, rabbit, horse, pig, monkey, rat, bovine, chicken and guinea pig.

**Summary** CD79a and b are the first components of BCR that are expressed developmentally. These subunits appear in association chaperone calnexin on pro-B cells. Subsequently, in pre-B cells, CD79 heterodimer is associated with lambda5-VpreB surrogate immunoglobulin and later with antigen-specific surface immunoglobulins. CD79a/b complex interacts with Src-family tyrosine kinase Lyn, which phosphorylates its cytoplasmic ITAM motives. The antibody HM57 recognizes the cytoplasmatic part of the CD79a protein, therefore the cells have to fixed and permeabilized in order to make the protein accessible for the antibody.

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

Membrane permeabilization (Starfiqs (IQP-200)) is required for this application.

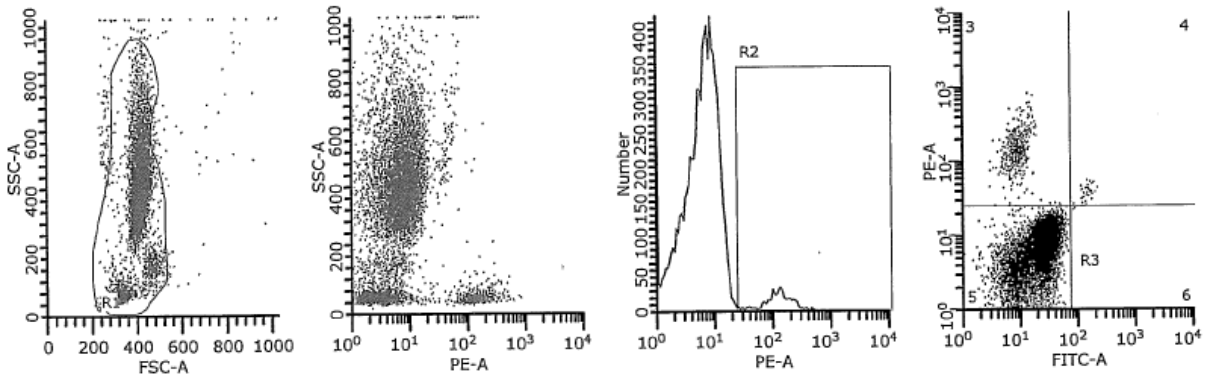
**Applications** CD79a (HM57) can be applied in flow cytometry for analysis of blood samples and immunohistochemistry on cryo slides.

**HLDA Workshop**

5th Leukocyte Typing Workshop, Schlossman S. et al. (Eds.), Oxford University Press (1995) and 6th Leukocyte Typing VI., Kishimoto T. et al. (Eds.), Garland Publishing Inc. (1997).

**Representative Data**

Staining with clone HM57 (CD79a) monoclonal antibodies is illustrated by flow cytometry analysis of normal blood cells. Direct staining was performed using 10 µl of the R-PE-conjugated antibody and 100 µl of a fixed and permeabilized blood sample.



**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% paraformaldehyde solution in PBS (store at 2 to 8 °C in amber glass for up to 1 week)

**IQ Starfiqs™: intracellular staining using flow cytometry**

Protocol for immuno-fluorescence staining of intracellular antigens

**IQ Starfiqs™** is a fixation and permeabilization solution intended for preparation of blood leukocytes before flow cytometry analysis of intracellular antigens. **IQ Starfiqs™** is a **ready to use** product, composed of two reagents used sequentially. The composition of both reagents is adjusted to ensure an optimum performance in flow cytometry analysis. Both reagents should be stored at 4 to 8 °C till the expiration period as indicated.

For optimal intracellular immunostaining and lysing of erythrocytes, **IQ Starfiqs™** should be used following the complete procedure as indicated below (see protocol). **IQ Starfiqs™** enables the detection of intracellular antigens such as CyCD3, CyCD22, TdT and MPO (myeloperoxidase).
In addition, the application of **IQ Starfiqs™** allows the simultaneous detection of cell surface antigens (see extended protocol **IQ Starfiqs™**). 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 IQ Starfiqs™ (staining of intracellular antigens)**

- Add 100 µl EDTA treated whole blood (bone marrow sample, mononuclear cell suspension) to a reagent tube.
- Add 100 µl **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs™** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent, or 20 µl of antibody conjugate for dual reagent.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

**Extended Protocol IQ Starfiqs™ (staining of cell surface antigens and intracellular antigens)**

- Add antibody conjugate to a reagent tube: 10 µl of antibody conjugate for single reagent directed against a cell surface antigen.
- Add 100 µl of EDTA- or Heparin-treated whole blood and mix well.
- Incubate for 15 minutes at room temperature in the dark.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant.
- Add 100 µl **IQ Starfiqs™** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs™** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent directed against an intracellular antigen.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

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**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 ($NaN_3$). Store the vials at 2 to 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.



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**References**

1. Mason, D.Y. et al. (1991) The IgM-associated protein mb-1 as a marker of normal and neoplastic B cells. J Immunol.147:2474 – 2482.
2. Jones, M. et al. (1993) Detection of T and B cells in many animal species using cross reactive and peptide antibodies. J Immunol. 150:5429 – 5435.
3. Mason DY, et al: CD79a: a novel marker for B-cell neoplasms in routinely processed tissue samples. Blood. 1995 Aug 15;86(4):1453-9.

**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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