# PRODUCT INFORMATION SHEET

Monoclonal antibodies detecting human antigens

## CD19

| | | | | |
|---|---|---|---|---|
| PURE | RUO | REF | IQP-515P | 100 tests |
| FITC | IVD | REF | IQP-515F | 100 tests |
| R-PE | IVD | REF | IQP-515R | 100 tests |
| CyQ | IVD | REF | IQP-515C | 100 tests |
| APC | IVD | REF | IQP-515A | 100 tests |
| PerCP | RUO | REF | IQP-515PC | 100 tests |
| PerCP-Cy5.5 | RUO | REF | IQP-515PCC | 100 tests |

IVD CE ***In Vitro Diagnostic medical device***
RUO ***For Research Use Only***

### Description

**Clone** HD37

**Isotype** murine IgG1

**Specificity** CD19 recognizes a 95 kD transmembrane glycoprotein.

**Antigen distribution**
CD19 (HD37) is present on all peripheral blood B cells. CD19 is also expressed on precursor B cells during maturation, but not on mature plasma cells.

**Summary** The function of the CD19 molecule is related to signal transfer and is involved in regulation of B cell proliferation. CD19 is considered to be a characteristic B cell marker and therefore commonly used in routine immunophenotyping. CD19 may also be expressed on follicular dendritic cells.

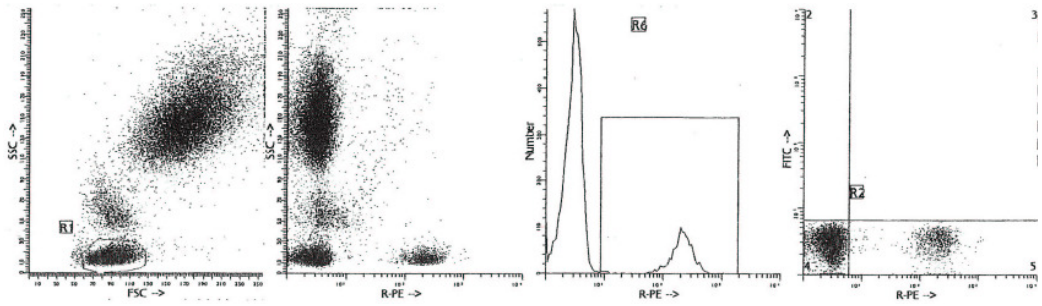
**Usage** All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µl/$10^6$ leukocytes for singles and 20 µl/$10^6$ leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate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

**Applications** CD19 (HD37) can be applied in flow cytometry and in immunohistochemistry using frozen tissue sections. Progenitor B cells mature in bone marrow and subsequently appear as mature CD19+ B cells in blood. After contact with foreign antigens and the appropriate T cell help, they may further differentiate to specific antibody-producing plasma cells. Detection of CD19 expressing cells is important in the diagnosis of leukemic precursor B cells (pre-B ALL), mature B cells (B ALL), and plasma cells. Distinction between subtypes of these (acute) leukemias can be performed using CD19 antibodies together with monoclonal antibodies to cytoplasmic or membrane Ig. A large number of B cell disorders can be effectively characterized by expression of CD19 and one or more additional antigens. One example is hairy cell leukemia (HCL), which shows specific expression of CD11c, CD19, CD20 and CD103. The combination CD103/CD19 is an important tool for diagnosis of HCL. Other valuable antibody combinations for distinction between different leukemias using CD19 antibodies are CD5/CD19 (e.g. Chronic Lymphatic Leukemia); CD10/CD19 (common ALL and pre-B ALL) and CD19/cyCD79a (Acute B cell leukemia).

**HLDA Workshop**
4th Leukocyte Typing Workshop - Knapp, W., et al., Oxford University Press, New York (1990)

## Representative Data

Staining with clone HD37 (CD19) 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 with 100 µl blood sample.




## 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 |
|---|---|---|---|---|---|
| CD19 FITC | 9 | 10.80 | 2.14 | 19.79 | IQP-515F |
| CD19 R-PE | 9 | 10.57 | 2.06 | 19.49 | IQP-515R |
| CD19 CyQ | 9 | 10.62 | 2.27 | 21.39 | IQP-515C |
| CD19 APC | 9 | 9.16 | 1.95 | 21.31 | IQP-515A |

## 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 2-8 °C in amber glass for up to 1 week)

**Immunofluorescence staining and lysing protocol**

*- A - Flow cytometry method for use with purified monoclonal antibodies*

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.
2. Add to each tube 10 µl of purified monoclonal antibody*. Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Wash the labeled cells by adding 2 ml of PBS containing 0.001% ($^v/_v$) Heparin, vortexing and centrifuging (2 min 1000 x g.) and discard the supernatant.
5. Add 50 µl of 1:10 dilution of IQ Products $F(ab)_2$ Rabbit Anti Mouse IgG fluorescent conjugate, [FITC (IQP-190F); R-PE (IQP-190R)] in PBS containing 0.001% ($^v/_v$) Heparin to the tube. It is recommended that the tube is protected from light.
6. Mix by vortexing and incubate for 15 minutes at room temperature in the dark.
7. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately.
8. Incubate for 10 minutes at room temperature in the dark.
9. Add 2 ml of demineralized water and incubate for 10 minutes in the dark
10. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
11. Remove the supernatant and resuspend the cells in 200 µl of PBS.**
12. 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).

*- B - Flow cytometry method for use with labeled (FITC, R-PE, CyQ, APC, PerCP or PerCP-Cy5.5) monoclonal antibodies*

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.
2. Add to each tube 10 µl of labeled monoclonal antibody*. Vortex the tube to ensure thorough mixing of antibody and cells.
3. Incubate the tube for 15 minutes at room temperature in the dark.
4. Add 100 µl of IQ Lyse (IQP-199 ready-to-use) and mix immediately.
5. Incubate for 10 minutes at room temperature in the dark.
6. Add 2 ml of demineralized water and incubate for 10 minutes in the dark.
7. Centrifuge the labeled cell suspension for 2 minutes at 1000 x g.
8. Remove the supernatant and resuspend the cells in 200 µl of PBS.**
9. 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).

*- C - 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) 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 2-8 °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.

## References


1. Poppema, S. and Visser, L., In: Monoclonal antibodies in the Characterization of Lymphomas and the Diagnosis of Disease; Proc. of the 9th Biotest Symposium, Institute of Education, London, 1987. Sonneborn H.H. and Tills D. Eds
2. Moldenhauer, G., et al., In Leucocyte Typing II Human B Lymphocytes, 1986, 61-67, E.L. Reinherz, B.F. Haynes, L.M. Nadler, and I.D. Bernstein eds. (Springer-Verlag, New York)
3. Meeker, T.C., et al., 1984. Hybridoma. 3: 305.
4. Loken, M.R., et al. 1987. Blood 70: 1316
5. Rothe, G., and Schmitz, G., Leukemia, 1996, 10: 877-895.
6. Leucocyte Typing IV 1990, Knapp, W. et al, eds. Oxford University Press.


## Explanation of used symbols

| | |
|---|---|
| | Consult instructions for use |
| REF | Catalogue number |
| Σ | 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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