## Intracellular Markers for Flow Cytometry

When intracellular antigens are key for relevant results.



Intracellular staining in flow cytometry is a useful technique in analysis of intracellular antigens. It has emerged as a powerful tool in the study of immunological signaling and related cellular processes.

## Antibodies useful for identifying intracellular antigens

**CD3:** Human TCR/CD3 is a complex structure on the lymphocyte surface. It consists of the TCR $\alpha\beta$  or TCR $\gamma\delta$  heterodimer and the associated CD3 complex. The CD3 complex is crucial in transducing antigen-recognition signals into the cytoplasm of T cells and in regulating the cell surface expression of the TCR complex.

**CD22:** CD22 is a single chain type 1 transmembrane glycoprotein, member of the IgG superfamily and homologous to several other proteins, including myelin basic protein and members of the carcinoembryonic antigen (CEA) family. CD22 expression is restricted to B cells and is absent from other haemopoietic cell types. The antigen is lost during the terminal stages of differentiation prior to the plasma cell stage.

**CD68:** CD68 belongs to a family of lysosomal glycoprotein (LGP)/plasma membrane shuttling proteins that play a role in endocytosis and/or lysosomal trafficking. CD68 is expressed strongly in cytoplasmic granules, and weakly on the surface of macrophages, monocytes, neutrophils, basophils and NK-cells.

**CD79acy:** CD79 is non-covalently associated with surface Ig, forming the B-cell receptor complex, which is required for antigen recognition. In precursor B cells, the CD79 protein chains are already expressed in the cytoplasm (CyCD79). Surface expression of CD79 begins at the pro-B cell stage and persists throughout the B-cell differentiation.

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**IgD, IgG and IgM:** Most B cells, with the exception of pre-B progenitor and pre-B cells, and mature plasma cells, express immunoglobulin on their surface. Pre-B cells express cyto-plasmic mu-chains but no light chains, whereas the early B lymphocytes express membrane IgM only. The maturing B lymphocytes additionally produce IgD that is inserted into the cell membrane joining IgM and establishing a population of IgM+IgD+ B lymphocytes, which is the largest population of circulating B lymphocytes in man.

Kappa and Lambda Light Chains: Most B cells, with the exception of pre-B progenitors and pre-B cells, and mature plasma cells, express immunoglobulin on their surface. Each cell expresses only one light chain type. In normal peripheral blood and lymph nodes, there is a mixture of kappa-positive and lambda-positive cells, with two-thirds of the cells expressing kappa and one-third expressing lambda.

Lysozyme: Within the haematopoietic system, lysozyme is known as an intracellular pan-myeloid marker molecule that is selectively expressed by cells of the granulo-monocytic lineage. Mature monocytes are thought to continuously synthesize and secrete lysozyme while granulocytes do not synthesize but only secrete the preformed enzyme.

**Myeloperoxidase (MPO):** Human myeloperoxidase (MPO) is a lysosomal enzyme present in the azurophilic granules of neutrophils and at lower levels in monocytes.

**Plasma Cell:** Anti-Human Plasma Cell, VS38c, was clustered as an anti-p63 protein. The p63 protein has a currently unknown function, but because of its homology to both rat and swine proteins, its abundance in secretory cells, and its localization to the rough endoplasmic reticulum, a conserved role in protein processing or secretion is suggested.

**Terminal Deoxynucleotidyl Transferase (TdT):** Terminal deoxynucleotidyl Transferase (TdT) catalyzes the random addition of deoxynucleotidyl residues on the 3' hydroxyl end of single-stranded DNA. TdT is present in the nuclei of T and B lymphocyte precursors.

## Intracellular Markers for Flow Cytometry, ASR\*

|     | Product                                       | Clone  | APC       | FITC      | PerCP     | PerCP-Cy5.5 | RPE       | РВ        |
|-----|---|--------|-----------|-----------|-----------|-------------|-----------|-----------|
| ASR | Mo a Hu CD3                                   | UCHT1  |           |           | PR70201-1 |             |           | PB98201-1 |
| ASR | Mo a Hu CD22                                  | 4KB128 | C728101-1 | F706001-1 |           | PR70750-1   | R706101-1 |           |
| ASR | Mo a Hu CD68                                  | KP1    |           | F713501-1 |           |             |           |           |
| ASR | Mo a Hu CD79αcy                               | HM57   | C725201-1 |           |           |             | R715901-1 |           |
| ASR | Rb a Hu IgD, Specific for Delta-Chains        |        |           | F018901-1 |           |             | R511201-1 |           |
| ASR | Rb a Hu IgG, Specific for Gamma-Chains        |        |           | F018501-1 |           |             |           |           |
| ASR | Rb a Hu IgM, Specific for Mu-Chains           |        |           | F005801-1 |           |             | R511101-1 |           |
| ASR | Rb a Hu Kappa Light Chains                    |        | C022201-1 | F043401-1 |           |             | R043601-1 |           |
| ASR | Rb a Hu Lambda Light Chains                   |        |           | F043501-1 |           | PR71250-1   | R043701-1 |           |
| ASR | Rb a Hu Lysozyme EC 3.2.1.17                  |        |           | F037201-1 |           |             |           |           |
| ASR | Mo a Hu Myeloperoxidase                       | MP0-7  | C724601-1 | F071401-1 |           | PR70450-1   | R720901-1 |           |
| ASR | Mo a Hu Plasma Cell                           | VS38c  |           | F714901-1 |           | PR71350-1   |           |           |
| ASR | Mo a Hu Terminal Deoxynucleotidyl Transferase | HT-6   |           | F713950-1 |           |             |           |           |
|     |   |        |           |           |           |             |           |           |

\* ASR: Analyte specific reagent. Analytical and performance characteristics are not established. Reagent provided consists of fluorochrome conjugated antibody that has been affinity isolated.

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