

## Cytek<sup>®</sup> cFluor<sup>®</sup> MDSC Kit

Myeloid-derived suppressor cells (MDSCs) are neutrophils and monocytes that possess potent immunosuppressive activity. The Cytek<sup>®</sup> cFluor<sup>®</sup> MDSC Kit has been designed and optimized to identify and analyze MDSCs. This kit includes markers to identify MDSCs and its subsets monocytic MDSC (M-MDSC) and granulocytic/polymorphonuclear MDSC (PMN-MDSC). A few newly identified MDSC markers such as, CD84, LOX-1, and CD181, could help to delineate the MDSC subsets and may also serve as indicators of cell status. MDSCs have been shown to play an important role in regulating immune responses in cancer and other pathological conditions, including chronic infection, sepsis, and autoimmunity. This kit can be a powerful tool to help researchers in their translational research and drug discovery.

| PRODUCT DETAILS FOR THE CYTEK® cFLUOR® MDSC PANEL |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
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| Catalog number:                                   | R7-40010 (25 Tests)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| Category:                                         | Immunoprofiling                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| Format:                                           | cFluor® conjugated antibodies in individual vials                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                          |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
|                                                   | <table><tr><th>Target</th><th>Clone</th><th>Fluorochrome</th></tr><tr><td>CD16</td><td>3G8</td><td>cFluor® V450</td></tr><tr><td>CD15</td><td>W6D3</td><td>cFluor® V505</td></tr><tr><td>CD14</td><td>MEM-18</td><td>cFluor® B515<sup>1</sup></td></tr><tr><td>CD45</td><td>HI30</td><td>cFluor® B548<sup>1</sup></td></tr><tr><td>CD84</td><td>CD84.1.21</td><td>cFluor® BYG575</td></tr><tr><td>CD11b</td><td>ICRF44</td><td>cFluor® BYG610<sup>1,2</sup></td></tr><tr><td>CD193 (CCR3)</td><td>5E8</td><td>cFluor® BYG667<sup>2</sup></td></tr><tr><td>CD181</td><td>8F1/CXCR1</td><td>cFluor® BYG710<sup>2</sup></td></tr><tr><td>CD33</td><td>WM53</td><td>cFluor® BYG781<sup>2</sup></td></tr><tr><td>LOX-1</td><td>15C4</td><td>cFluor® R659</td></tr><tr><td>CD3</td><td>SK7</td><td rowspan="3">cFluor® R685<sup>1</sup></td></tr><tr><td>CD19</td><td>HIB19</td></tr><tr><td>CD56</td><td>TULY56</td></tr><tr><td>CD66b</td><td>G10F5</td><td>cFluor® R720<sup>1</sup></td></tr><tr><td>HLA-DR</td><td>L243</td><td>cFluor® R840<sup>1,2</sup></td></tr></table> |                               | Target | Clone | Fluorochrome | CD16 | 3G8 | cFluor® V450 | CD15 | W6D3 | cFluor® V505 | CD14 | MEM-18 | cFluor® B515 <sup>1</sup> | CD45 | HI30 | cFluor® B548 <sup>1</sup> | CD84 | CD84.1.21 | cFluor® BYG575 | CD11b | ICRF44 | cFluor® BYG610 <sup>1,2</sup> | CD193 (CCR3) | 5E8 | cFluor® BYG667 <sup>2</sup> | CD181 | 8F1/CXCR1 | cFluor® BYG710 <sup>2</sup> | CD33 | WM53 | cFluor® BYG781 <sup>2</sup> | LOX-1 | 15C4 | cFluor® R659 | CD3 | SK7 | cFluor® R685 <sup>1</sup> | CD19 | HIB19 | CD56 | TULY56 | CD66b | G10F5 | cFluor® R720 <sup>1</sup> | HLA-DR | L243 | cFluor® R840 <sup>1,2</sup> |
| Target                                            | Clone                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | Fluorochrome                  |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD16                                              | 3G8                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | cFluor® V450                  |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD15                                              | W6D3                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | cFluor® V505                  |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD14                                              | MEM-18                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | cFluor® B515 <sup>1</sup>     |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD45                                              | HI30                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | cFluor® B548 <sup>1</sup>     |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD84                                              | CD84.1.21                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | cFluor® BYG575                |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD11b                                             | ICRF44                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     | cFluor® BYG610 <sup>1,2</sup> |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD193 (CCR3)                                      | 5E8                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | cFluor® BYG667 <sup>2</sup>   |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD181                                             | 8F1/CXCR1                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  | cFluor® BYG710 <sup>2</sup>   |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD33                                              | WM53                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | cFluor® BYG781 <sup>2</sup>   |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| LOX-1                                             | 15C4                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | cFluor® R659                  |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD3                                               | SK7                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                        | cFluor® R685 <sup>1</sup>     |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD19                                              | HIB19                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD56                                              | TULY56                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                     |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| CD66b                                             | G10F5                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                      | cFluor® R720 <sup>1</sup>     |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| HLA-DR                                            | L243                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                       | cFluor® R840 <sup>1,2</sup>   |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| Test Dilution:                                    | 5 µl per test                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                              |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| Application:                                      | Flow cytometry                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                             |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| Formulation:                                      | Phosphate-buffered saline, pH 7.2, containing 0.09% sodium azide and 0.2% BSA (BSA Country of Origin USA)                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                  |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |
| Storage:                                          | 2-8°C and protected from light.<br>Do not freeze                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                           |                               |        |       |              |      |     |              |      |      |              |      |        |                           |      |      |                           |      |           |                |       |        |                               |              |     |                             |       |           |                             |      |      |                             |       |      |              |     |     |                           |      |       |      |        |       |       |                           |        |      |                             |

## PRODUCT DESCRIPTION

The Cytex® cFluor® MDSC Kit identifies MDSC subsets, M-MDSC and PMN-MDSC, and monocyte subsets, classical, intermediate, and non-classical monocytes. The kit can be used on whole blood and peripheral mononuclear cells (PBMC).

**CD3** is expressed on all mature T cells, NKT cells and thymocytes. It plays a role in recognizing antigen, activating cytotoxic T cell and T helper cell, and signal transduction.

**CD11b** is a type I transmembrane glycoprotein also known as αM integrin, Mac-1, CR3, and C3biR. CD11b associated with integrin β2 (CD18) is expressed on the surface of monocytes, granulocytes, activated lymphocytes and a subset of NK cells. CD11b is a receptor for intercellular adhesion molecule family members CD54, CD102 and CD50 as well as for iC3b. These adhesions are crucial in cell-cell and cell-matrix interactions.

**CD14** is a glycosylphosphatidylinositol (GPI)-linked membrane glycoprotein that works as a receptor on myeloid cells for ligands such as lipopolysaccharide (LPS). CD14 is a receptor for and binds to complexes of LPS and LPS-binding protein (LBP) with high affinity. It expresses high levels on monocytes, also expressed on interfollicular macrophages, reticular dendritic cells and Langerhans cells. It is also found on granulocytes.

**CD15** is also known as 3-fucosyl-N-acetylglucosamine (3-FAL), Lewis X, 3-FAL, X-hapten, and SSEA-1. It is highly expressed on granulocytes, including neutrophils and eosinophils, and some on monocytes. It is absent from lymphocytes or basophils. CD15, a marker for human myeloid cell, is involved in various cell functions including phagocytosis, bacterial activity, neutrophil adhesion to dendritic cells and chemotaxis.

**CD16** is in the form of CD16a and CD16b. CD16a is expressed on NK cells and macrophages while CD16b is expressed on neutrophils. CD16a also plays a crucial role for antibody-dependent cellular cytotoxicity (ADCC) by NK cells.

**CD19** forms a signal transduction complex with the complement receptor 2 (CD21), a tetraspanin membrane protein, TAPA-1 (CD81), and Lue 13 to function as a dominant signaling component on the surface of B cells. It is expressed in all phases of B cell development, maturation, and differentiation except at the terminal stage of differentiation, lost in plasma cells. It is also present on follicular dendritic cells and absent on T cells.

**CD33** also known as Siglec-3, gp67, and p67, is expressed on monocytes, activated T cells, myeloid progenitors, granulocytes, dendritic cells, and mast cells. It is absent on erythrocytes, platelets, and lymphoid cells. CD33 binds to sialic acids to act as a sialic acid-dependent cell adhesion molecule. It also contains intracellular tyrosine-based inhibition motifs (ITIMs), suggesting its function to inhibit cellular activity.

**CD45** is the first and prototypic receptor-like protein tyrosine phosphatase that expresses on all human leukocytes. It is absent on mature erythrocytes, platelets, and non-hematopoietic cells.

**CD56**, also known as NCAM (Neural Cell Adhesion Molecule), Leu-19 and NKH1, is present on NK and NKT cells. CD56 is also expressed in the brain (cerebellum and cortex) and at neuromuscular junctions. Aberrant CD56 expression is observed in a range of hematological malignancies such as multiple myeloma and leukemia as well as in solid tumors such as lung cancer, ovarian cancer, and neuroblastoma.

**CD66b**, is a member of the carcinoembryonic antigen (CEA)-like subfamily. CD66b, expressed on granulocytes, has been reported to induce activation in neutrophils and to be involved in heterophilic adhesion with CD66c.

**CD84** is a member of the SLAM (CD150) family, also known as SLAMF5 or Ly9b. CD84 is expressed on B cells, monocytes, thymocytes, subset of T cells (preferentially CD45RO<sup>+</sup> T cells), and platelets. CD84 functions as a homophilic adhesion molecule and enhances T cell activation and cytokine production.

**CD181** also known as CXCR1, IL-8 receptor A (IL-8RA), and CDw128a. It is a CXC chemokine receptor that belongs to the G protein-coupled receptor (GPCR) family. CXCR1 is expressed as a homodimer or heterodimer with CXCR2 and found on granulocytes, NK cells, subsets of T lymphocytes, mast cells, monocytes, endothelial cells, megakaryocytes, and oligodendrocytes. CXCR1 mediates neutrophil activation and chemotaxis, megakaryocytic proliferation, and angiogenesis.

**CD193 (CCR3)** is a member of the G protein-coupled seven transmembrane receptors family, and binds to the CC chemokines eotaxin, eotaxin-2, and eotaxin-3 with high affinity. It has also been reported to bind RANTES, MCP-3, and MCP-4 with low affinity. CCR3 receptor is expressed on human eosinophils, basophils, mast cells, mononuclear phagocytes, platelets, CD34<sup>+</sup> hematopoietic progenitor cells, Th2-like lymphocytes, and keratinocytes. CCR3 is thought to play a role in allergic diseases such as bronchial asthma and allergic rhinitis. CCR3 is a co-receptor for HIV-1 and HIV-2, and the binding of eotaxin with CCR3 has been shown to inhibit HIV infection in some cell types.

**HLA-DR**, also known as human leukocyte antigen DR isotype, is present on the surface of antigen-presenting cells, including B cells, dendritic cells, macrophages, monocytes, and activated T cells. MHC class II regulates the immune system by playing a critical role in binding and presenting antigen-derived peptides to peptide-MHC II-specific CD4 T cells.

**LOX-1** is expressed by endothelial cells, smooth muscle cells, platelets, fibroblasts, and macrophages and is upregulated by inflammatory and oxidative stimuli. LOX-1 is involved in endocytosis, phagocytosis and cytokine production.

## RECOMMENDED USAGE

Whole blood collected in K<sub>2</sub>EDTA, Heparin, ACD, and Cyto-Chex® BCT blood tubes have been tested to validate the performance of this kit. PBMCs and tissue infiltrating leukocytes have also been tested and validated. Please refer to the product web page for the staining protocols, fluorochrome list, experiment template and data acquisition protocol.

Please briefly centrifuge the reagent vial before use.

Use appropriate personal protective equipment per the product safety data sheet when using this product.

## REFERENCES

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<sup>1</sup> cFluor® B515, cFluor® B548, cFluor® BYG610, cFluor® R685, cFluor® R720, and cFluor® R840 are equivalent to CF®488A, CF®514, RPE-CF®596R, CF®660C, CF®700, and APC-CF®790T respectively, manufactured and provided by Biotium, Inc. under an Agreement between Biotium and Cytek (LICENSEE). The manufacture, use, sale, offer for sale, or import of the product is covered by one or more of the patents or pending applications owned or licensed by Biotium. The purchase of this product includes a limited, non-transferable immunity from suit under the foregoing patent claims for using only this amount of product for the purchaser's own internal research. No right under any other patent claim, no right to perform any patented method, and no right to perform commercial services of any kind, including without limitation reporting the results of purchaser's activities for a fee or other commercial consideration, is conveyed expressly, by implication, or by estoppel.

<sup>2</sup> cFluor® BYG610, cFluor® BYG667, cFluor® BYG710, and cFluor® BYG781 are tandem dyes made with R-PE. cFluor® R840 is a tandem dye made with APC. Caution – Tandem dyes may show changes in their emission spectra with prolonged exposure to light or fixatives.

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