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# Suppressive Myeloid Cell Phenotyping IHC Antibody Sampler Kit



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New 10/19

For Research Use Only. Not For Use In Diagnostic Procedures.

Products Included	Product #	Quantity	Isotype/Source
CD14 (D7A2T) Rabbit mAb (IHC Formulated)	75181	20 µl	Rabbit IgG
CD11b/ITGAM (D6X1N) Rabbit mAb	49420	20 µl	Rabbit IgG
CD68 (D4B9C) XP® Rabbit mAb	76437	20 µl	Rabbit IgG
CD163 (D6U1J) Rabbit mAb	93498	20 µl	Rabbit IgG
CD206/MRC1 (E2L9N) Rabbit mAb	91992	20 µl	Rabbit IgG
M-CSF Receptor (E4T8Z) Rabbit mAb	28917	20 µl	Rabbit IgG
Arginase-1 (D4E3M™) XP® Rabbit mAb	93668	20 µl	Rabbit IgG
MHC Class II (LGII-612.14) Mouse mAb	68258	20 µl	Mouse IgG1
CD15/SSEA1 (MC480) Mouse mAb	4744	20 µl	Mouse IgM

See [www.cellsignal.com](http://www.cellsignal.com) for individual component applications, species cross-reactivity, dilutions, and additional application protocols.

**Background:** A combination of multiple biomarkers are required to characterize the phenotype of myeloid cell lineages. Cluster of differentiation molecule 14 (CD14) is a leucine-rich repeat-containing pattern recognition receptor with expression largely restricted to the monocyte/macrophage cell lineage (1), but can be unregulated on polymorphonuclear as well as nonmyeloid cells such as B cells and gingival fibroblasts (2,3). CD11b (Integrin alpha M or ITGAM) is a transmembrane protein forming heterodimers that are composed of  $\alpha$  and  $\beta$  subunits (4). CD11b is expressed by, and commonly used as a marker for myeloid lineage cells, including neutrophils, monocytes, macrophages, dendritic cells, and microglia (5), but has also been detected on a subset of B cells (6-8). CD68 (macrosialin) is a heavily glycosylated transmembrane protein that is expressed by and commonly used as a marker for monocytes and macrophages (9,10), but there is also evidence of non-myeloid cell expression (11). The CD15 carbohydrate epitope is preferentially expressed in mature human neutrophils, monocytes, and all myeloid cells from the promyelocyte stage onwards, making it a useful cell surface marker (12-14). It is also expressed in some tissues, such as epithelial cells of intestinal tissues (15,16), and in certain neurons and glial cells in the central nervous system (17).

CD163 is a transmembrane scavenger receptor expressed on the macrophage surface. It has 9 B-type SRCR extracellular domains mediating serum haptoglobin clearing/endocytosis, pathogen binding and signal transduction, and calcium binding (18,19). The mannose receptor (CD206/MR/CLEC13D/MMR/MRC1/Macrophage mannose receptor 1) is an endocytic receptor expressed by populations of dendritic cells, macrophages, and nonvascular endothelium (20). CD206/MRC1 receptor functions include a role in antigen cross-presentation, clearance of endogenous proteins, pathogen detection and trafficking through lymphatic vessels (21-24). Macrophage-colony stimu-

lating factor (M-CSF, CSF-1) receptor is an integral membrane tyrosine kinase encoded by the *c-fms* proto-oncogene. M-CSF receptor is expressed in monocytes (macrophages and their progenitors) and drives growth and development of this blood cell lineage (25,26). CD163, CD206, and M-CSF receptors are used as surface markers of M2 type macrophages, including M2 type tumor associated macrophages (TAMs), which facilitate cancer progression by secreting cytokines to promote angiogenesis, immunosuppression, and metastasis (20,27,28). Arginase-1 catalyzes the final step of the urea cycle converting L-arginine to L-ornithine and urea (29). Myeloid-derived suppressor cells express high levels of arginase-1, increasing the catabolism of L-arginine resulting in L-arginine depletion in the inflammatory microenvironment of cancer. The reduced availability of L-arginine suppresses T cell proliferation and function and thus contributes to tumor progression (30,31).

Major histocompatibility complex class II (MHC class II) molecules are heterodimeric, transmembrane glycoproteins expressed on the surface of antigen-presenting cells such as macrophages, dendritic cells, and B cells. Expression can also be induced through interferon- $\gamma$  signaling (32). Prior to being displayed on the cell membrane, MHC class II molecules are loaded with exogenous peptide antigens approximately 15-24 amino acids in length that were derived from endocytosed extracellular proteins digested in the lysosome. Antigen-presentation through MHC class II is required for T cell activation during the immune response to extracellular pathogens (33). High expression of MHC class II on myeloid cell lineages is used as a surface marker of M1 type macrophages, including M1 type TAMs, which can assist in tumor eradication by secreting cytokines to activate anti-tumor immune responses, and inhibit angiogenesis and metastasis (27,28).

**Storage:** Supplied in 10 mM sodium HEPES (pH 7.5), 150 mM NaCl, 100 µg/ml BSA, 50% glycerol and less than 0.02% sodium azide. Store at -20°C. Do not aliquot the antibodies.

Please visit [www.cellsignal.com](http://www.cellsignal.com) for validation data and a complete listing of recommended companion products.

**Product Description:** The Suppressive Myeloid Cell Phenotyping IHC Antibody Sampler Kit provides an economical means of detecting the accumulation of immune cell types in formalin-fixed, paraffin-embedded tissue samples.

**Specificity/Sensitivity:** Each antibody included in the Suppressive Myeloid Cell Phenotyping IHC Antibody Sampler Kit recognizes endogenous levels of its target human protein. M-CSF Receptor (E4T8Z) Rabbit mAb cross-reacts with an unidentified protein of 70 kDa in some cell extracts. Arginase-1 (D4E3M™) XP® Rabbit mAb does not cross-react with arginase-2. MHC Class II (LGII-612.14) Mouse mAb exhibits strong reactivity with HLA-DRB and weak reactivity with HLA-DPB in cell lines transfected with constructs expressing Myc/DDK-tagged HLA-DRB and HLA-DPB, respectively. Reactivity is not observed with HLA-DMB, HLA-DOB, and HLA-DQB in cell lines transfected with constructs expressing Myc/DDK-tagged HLA-DMB, HLA-DOB, and HLA-DQB.

**Source/Purification:** Monoclonal antibodies are produced by immunizing animals with synthetic peptides corresponding to residues surrounding Pro319 of human CD14 protein or Val47 of human arginase-1 protein, or with recombinant protein specific to human CD68 protein, human CD163 protein, human CD206/MRC1 protein, the carboxy terminus of human M-CSF receptor protein, or the amino terminus of human CD11b/ITGAM protein. CD15/SSEA1 (MC480) Mouse mAb is produced by immunizing animals with F9 teratoma cells (X-irradiated). MHC Class II (LGII-612.14) Mouse mAb is produced by immunizing animals with cultured human B lymphoid cells treated with IFN- $\gamma$ .

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**Applications:** W—Western IP—Immunoprecipitation IHC—Immunohistochemistry ChIP—Chromatin Immunoprecipitation IF—Immunofluorescence F—Flow cytometry E-P—ELISA-Peptide **Species Cross-Reactivity:** H—human M—mouse R—rat Hm—hamster Mk—monkey Mi—mink C—chicken Dm—D. melanogaster X—Xenopus Z—zebrafish B—bovine Dg—dog Pg—pig Sc—S. cerevisiae Ce—C. elegans Hr—Horse All—all species expected **Species enclosed in parentheses are predicted to react based on 100% homology.**

## Background References:

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