

# CD192 (CCR2) antibodies, mouse

## For research use only

9 μg equal 60 tests, 30 μg equal 200 tests. One test corresponds to labeling of 10<sup>6</sup> cells.

Product	Content	Order no.
CD192 (CCR2)-PE	9 μg in 300 μL	130-108-789
CD192 (CCR2)-PE	30 μg in 1 mL	130-108-722
CD192 (CCR2)-APC	9 μg in 300 μL	130-108-790
CD192 (CCR2)-APC	30 μg in 1 mL	130-108-723
CD192 (CCR2)-PE-Vio770	9 μg in 300 μL	130-108-791
CD192 (CCR2)-PE-Vio770	30 μg in 1 mL	130-108-724
CD192 (CCR2)-APC-Vio770	9 μg in 300 μL	130-108-792
CD192 (CCR2)-APC-Vio770	30 μg in 1 mL	130-108-725
CD192 (CCR2)-Biotin	9 μg in 300 μL	130-108-788
CD192 (CCR2)-Biotin	30 μg in 1 mL	130-108-721

## Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

#### Technical data and background information

Antigen CD192 (CCR2)

Clone REA538

Isotyperecombinant human IgG1Isotype controlREA Control antibodies

Alternative names of antigen CCR2, CC-CKR-2, CCR2B, Ckr2, CKR2A, Ckr2b, Cmkbr2,

mJe-r, MCP-1-R

Molecular mass of antigen [kDa] 43

**Distribution of antigen** macrophages, monocytes, T cells

Product format Reagents are supplied in buffer containing stabilizer and 0.05%

sodium azide.

**Fixation** Cells should be stained prior to fixation, if formaldehyde is used

as a fixative.

**Storage** Store protected from light at 2–8 °C. Do not freeze.

Clone REA538 recognizes the mouse CD192 antigen, a multi-pass membrane protein, which is also known as C-C chemokine receptor type 2 (CCR2) or monocyte chemoattractant protein 1 receptor (MCP-1-R). CD192 is a receptor for the CCL2, CCL7, and CCL12 chemokines and plays important roles in the recruitment of monocytes, macrophages, and T cells. It transduces a signal by increasing

intracellular calcium ion levels. In mice CD192 is homogeneously expressed on monocytes and on 2–15% of T cells, closely resembling the expression pattern in humans. The percentage of CD192<sup>+</sup> T cells is almost two times higher in the TH1-prone mouse strain C57BL/6 than in the TH2-prone strain BALB/c. The strain-dependent expression correlates to a reduced TH1 response described in CD192-deficient mice.

Additional information: Clone REA538 displays negligible binding to Fc receptors.

#### Reagent requirements

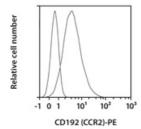
- Buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS<sup>®</sup> BSA Stock Solution (# 130-091-376) 1:20 with autoMACS<sup>®</sup> Rinsing Solution (# 130-091-222). Keep buffer cold (2-8 °C). Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). Buffers or media containing Ca<sup>2+</sup> or Mg<sup>2+</sup> are not recommended for use.
- (Optional) Fluorochrome-conjugated anti-biotin antibodies, e.g., Anti-Biotin-PE (# 130-090-756) as secondary antibody reagent in combination with biotinylated antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) for flow cytometric exclusion of dead cells without fixation.
- (Optional) Fixation and Dead Cell Discrimination Kit (# 130-091-163) for cell fixation and flow cytometric exclusion of dead cells.

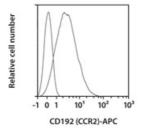
## Protocol for cell surface staining

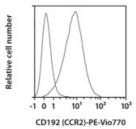
- The recommended antibody dilution for labeling of cells and subsequent analysis by flow cytometry is 1:10 for up to 10<sup>6</sup> cells/50 µL of buffer.
- Volumes given below are for up to 10<sup>6</sup> nucleated cells. When working with fewer than 10<sup>6</sup> cells, use the same volumes as indicated. When working with higher cell numbers, scale up all reagent volumes and total volumes accordingly (e.g. for 2×10<sup>6</sup> nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).
- 1. Determine cell number.
- 2. Centrifuge cell suspension at 300×g for 10 minutes. Aspirate supernatant completely.
- 3. Resuspend up to  $10^6$  nucleated cells per 45  $\mu$ L of buffer.
- 4. Add 5 μL of the antibody.
- 5. Mix well and incubate for 10 minutes in the dark in the refrigerator (2-8 °C).
  Note: Higher temperatures and/or longer incubation times may lead to non-specific cell labeling.
  Working on ice requires increased incubation times.
- 6. Wash cells by adding 1–2 mL of buffer and centrifuge at 300×g for 10 minutes. Aspirate supernatant completely.
- 7. (Optional) If biotinylated antibody was used, resuspend the cell pellet in 100  $\mu$ L of buffer, add 10  $\mu$ L of fluorochrome-conjugated anti-biotin antibody, and continue as described in steps 5 and 6.
- 8. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

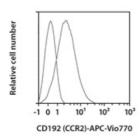
### **Examples of immunofluorescent staining**

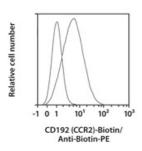
CD192 (CCR2) transfected cells were stained with CD192 (CCR2) antibodies or with the corresponding REA Control antibodies (left peak). Flow cytometry was performed using the MACSQuant<sup>®</sup> Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.











#### References

- Kurihara, T. et al. (1996) Cloning and functional expression of mCCR2, a murine receptor for the C-C chemokines JE and FIC. J. Biol. Chem. 271(20): 11603–11607.
- Mack, M. et al. (2001) Expression and characterization of the chemokine receptors CCR2 and CCR5 in mice. J. Immunol. 166(7): 4697–4704.
- 3. **Majmudar, M. D. et al.** (2013) Monocyte-directed RNAi targeting CCR2 improves infarct healing in atherosclerosis-prone mice. Circulation 127(20): 2038–2046.

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