



Antibodies

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1. Description

Clone	EP-1 (isotype: mouse IgG1).
Product format	1 mL Anti-Melanoma (MCSP) antibodies, human: monoclonal melanoma cell-specific antibodies conjugated to R-phycoerythrin (PE), or allophycocyanin (APC). The antibodies are supplied in a solution containing 0.1% gelatine and 0.05% sodium azide.
Product size	For 10 ⁹ total cells, up to 100 stainings.
Storage	Store protected from light at 4–8 °C. Do not freeze. The expiration date is indicated on the vial label.

1.1 Background and product applications

The Anti-Melanoma (MCSP) antibody recognizes the melanoma-associated chondroitin sulfate proteoglycan (MCSP) antigen, also known as high-molecular weight melanoma associated antigen. MCSP is a unique glycoprotein-proteoglycan complex consisting of an N-linked glycoprotein of 250 kDa and a proteoglycan component of > 450 kDa. It has been detected on melanoma cells but not on carcinoma cells, fibroblastoid cells and cells of hematopoietic origin¹⁻⁴.

Product applications

- Identification and enumeration of melanoma cells by flow cytometry or fluorescence microscopy.
- Evaluation of MACS[®] separations by flow cytometry or fluorescence microscopy. Melanoma cells can be isolated by using Anti-Melanoma (MCSP) MicroBeads (# 130-090-452).
- Detection of disseminated melanoma cells, e.g. in peripheral blood^{5,6} or bone marrow by flow cytometry or fluorescence microscopy.
- Analysis of single-cell suspensions of skin biopsies or primary skin cell cultures by flow cytometry or fluorescence microscopy.

Anti-Melanoma (MCSP) antibodies

human

Anti-Melanoma (MCSP)-PE	130-091-225
Anti-Melanoma (MCSP)-APC	130-091-252

1.2 Examples of staining concentrations

Anti-Melanoma-conjugate	PE	APC
Recommended antibody dilution*		
Flow cytometry		
- in general	1:11	1:11
- formaldehyde-fixed cells	1:11	1:11
- Anti-Melanoma MicroBead-labeled cells	1:11	1:11

* Given antibody dilutions are for a cell concentration of up to 1×10⁸ cells/mL buffer.

1.3 Reagent requirements

- Buffer: Prepare a solution containing PBS (phosphate buffered saline) pH 7.2, 0.5% BSA and 2 mM EDTA, e.g. by diluting MACS BSA Stock Solution (# 130-091-376) 1:20 with autoMACS[™] Rinsing Solution (# 130-091-222). Keep buffer cold (4–8 °C).
▲ Note: EDTA can be replaced by other supplements such as anticoagulant citrate dextrose formula-A (ACD-A) or citrate phosphate dextrose (CPD). BSA can be replaced by other proteins such as human serum albumin, human serum or fetal calf serum. Buffers or media containing Ca²⁺ or Mg²⁺ are not recommended for use.
- (Optional) PI (propidium iodide) or 7-AAD for flow cytometric exclusion of dead cells without cell fixation. For cell fixation and flow cytometric exclusion of dead cells, the Fixation and Dead Cell Discrimination Kit (# 130-091-163) is recommended.

2. General protocol for immunofluorescent staining

▲ Volumes for fluorescent labeling given below are for up to 10⁷ total cells. When working with fewer than 10⁷ 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 2×10⁷ total cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Resuspend 10⁷ cells in 100 µL of buffer.
2. Add 10 µL of Anti-Melanoma (MCSP) antibodies.
3. Mix well and incubate for 10 minutes in the dark at 4–8 °C.
▲ Note: Working on ice requires increased incubation times. Higher temperatures and/or longer incubation times lead to non-specific cell labeling.
4. Wash cells by adding 1–2 mL of buffer per 10⁷ cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.
5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

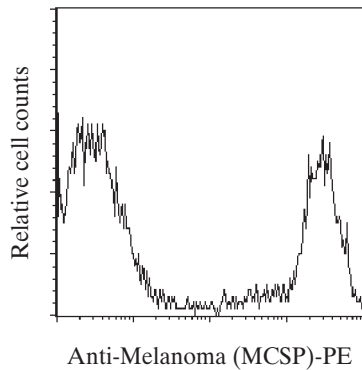
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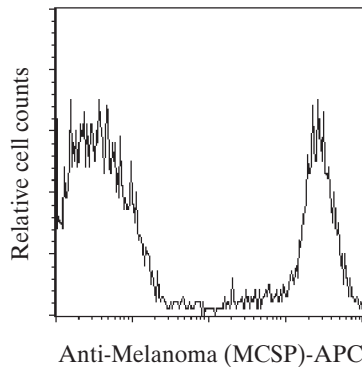
3. Examples of immunofluorescent staining with Anti-Melanoma (MCSP) antibodies

Human peripheral blood leukocytes mixed with cells from the melanoma cell line SK-MEL-28 were stained with Anti-Melanoma (MCSP) antibodies conjugated to PE (a), or APC (b), and analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.

(a) Human peripheral blood leukocytes mixed with SK-MEL-28 cells stained with Anti-Melanoma (MCSP)-PE.



(b) Human peripheral blood leukocytes mixed with SK-MEL-28 cells stained with Anti-Melanoma (MCSP)-APC.



4. References

1. Morgan *et al.* (1981) *Hybridoma* 1: 27–36.
2. Bumol and Reisfeld (1982) *Proc. Natl. Acad. Sci. USA* 79: 1245–1249.
3. Bumol *et al.* (1984) *J. Biol. Chem.* 259: 12733–12741.
4. Pluschke *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93: 9710–9715.
5. Benez *et al.* (1999) *J. Clin. Lab. Anal.* 13, 229–233.
6. Siewert *et al.* (2000) *Recent Results in Cancer Research* 158: 51–60

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.

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