Miltenyi Biotec

Anti-Cytokeratin (CK3-6H5) antibodies

human

Anti-Cytokeratin (CK3-6H5)-FITC	130-080-101
Anti-Cytokeratin (CK3-6H5) pure	130-090-866

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1. Description

Clone	CK3-6H5 (isotype: mouse IgG1).
Product format	1 mL Anti-Cytokeratin (CK3-6H5) antibodies, human: monoclonal Anti-Cytokeratin (CK3-6H5) antibodies conjugated to fluoresceinisothiocyanate (FITC). The unconjugated (pure) antibody is supplied at a concentration of 100 µg/mL. The antibodies are supplied in a solution containing stabilizer and 0.05% sodium azide.
Product size	100 tests or up to 10^9 total cells.
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

Cytokeratins are typical intermediate filaments of the cytoskeleton of epithelial cells. They are a biochemically highly diverse multigene family of polypeptides with molecular masses ranging from 40- to 68-kDa. Until now 20 distinct cytokeratin polypeptides have been described, which can be divided into an acidic (type I) and a neutral-basic (type II) subfamily. The cytokeratin expression profile within one cell characterizes the type of epithelia and also the degree of maturation or differentiation. Most malignant cells which have their origin in epithelial tissue express a certain cytokeratin profile, which can be used for classifying carcinomas and for distinguishing carcinomas from malignant tumors of non-epithelial origin. Anti-Cytokeratin (CK3-6H5) is a pancytokeratin-specific antibody recognizing probably all simple epithelium cytokeratins. It crossblocks Cam5.2, an antibody known to be specific for cytokeratins 7 and 8.

Product applications

- Detection and analysis of disseminated epithelial tumor cells in peripheral blood, bone marrow or lymphoid tissue, e.g. by flow cytometry or fluorescence microscopy.
- Immunofluorescent staining of disseminated epithelial tumor cells in frozen sectioned or formalin-fixed paraffin-embedded human tissue, e.g. primary tumor tissue.

1.2 Recommended antibody dilution

For antibody labeling of human cells.

Anti-Cytokeratin (CK3-6H5)-conjugate	FITC
Flow cytometry	
- in general	1:11
- formaldehyde-fixed cells	1:11
- Anti-Cytokeratin (CK3-6H5) MicroBead-labeled cells	1:11

a) Given antibody dilutions are for a cell concentration of up to 1×10^7 cells/mL of buffer.

1.3 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® 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 Ca^{2+} or Mg^{2+} are not recommended for use.
- Inside Stain Kit (# 130-090-477) or Carcinoma Cell Enrichment Kit (# 130-060-101) for fixation and permeabilisation.
- (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

▲ Direct immunofluorescent staining with Anti-Cytokeratin (CK3-6H5)-FITC can be performed on cells fixed with e.g. MACS Inside Fix or MACS Cell Fix, respectively. For detailed protocols, see Inside Stain Kit data sheet, or MACS Carcinoma Cell Enrichment Kit data sheet, respectively.

▲ Volumes for fluorescent labeling given below are for **up to 10^7** nucleated cells. When working with fewer than 10^7 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 2×10^7 nucleated cells, use twice the volume of all indicated reagent volumes and total volumes).

1. Dilute 10 µL Anti-Cytokeratin (CK3-6H5)-FITC in 90 µL MACS Inside Perm or MACS Cell Stain, respectively.
2. Add 100 µL of the diluted Anti-Cytokeratin (CK3-6H5)-FITC antibody per 10^7 fixed cells.
3. Mix well and incubate for 10 minutes in the dark at room temperature.

   ▲ **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 mL of MACS Inside Perm (or MACS Cell Stain, respectively) per 10^7 cells and centrifuge at 300×g for 10 minutes. Pipette off supernatant completely.

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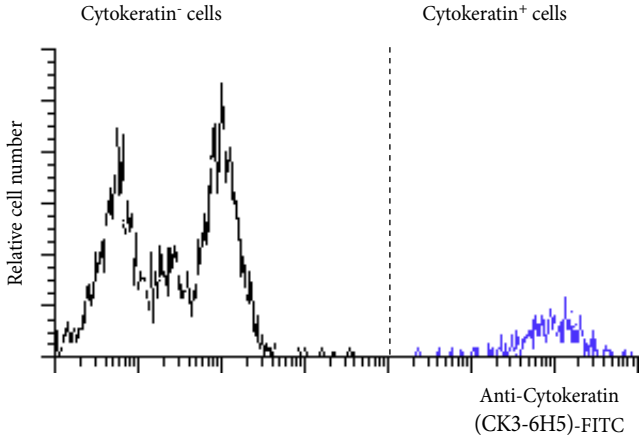
Miltenyi Biotec GmbH
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macs@miltenyibiotec.de
www.miltenyibiotec.com

Miltenyi Biotec Inc.
2303 Lindbergh Street, Auburn, CA 95602, USA
Phone 800 FOR MACS, +1 530 888 8871, Fax +1 530 888 8925
macs@miltenyibiotec.com

5. Resuspend cell pellet in a suitable amount of buffer for analysis by flow cytometry or fluorescence microscopy.

3. Examples of immunofluorescent staining with Anti-Cytokeratin (CK3-6H5) antibodies

Peripheral blood leukocytes mixed with cells from a breast cancer cell line (SK-BR-3) were permeabilized and fixed using MACS® Cell Fix solution and MACS Cell Perm solution from the MACS Carcinoma Cell Enrichment Kit. Cells were stained using staining buffer and Anti-Cytokeratin (CK3-6H5)-FITC and were analyzed by flow cytometry. Cell debris and dead cells were excluded from the analysis based on scatter signals and PI fluorescence.



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.

Warranty

The products sold hereunder are warranted only to be free from defects in workmanship and material at the time of delivery to the customer. Miltenyi Biotec GmbH makes no warranty or representation, either expressed or implied, with respect to the fitness of a product for a particular purpose. There are no warranties, expressed or implied, which extend beyond the technical specifications of the products. Miltenyi Biotec GmbH's liability is limited to either replacement of the products or refund of the purchase price. Miltenyi Biotec GmbH is not liable for any property damage, personal injury or economic loss caused by the product.

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