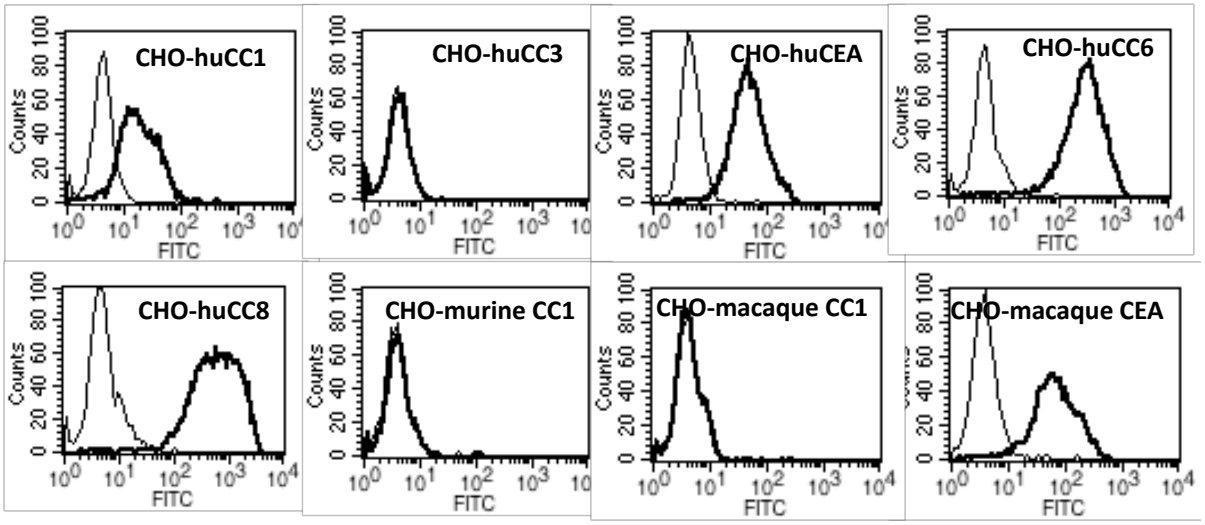
Leuko Com

anti human CEACAM1/5/6/8
monoclonal antibody
mouse IgG1; **clone 4H6-20**

DESCRIPTION

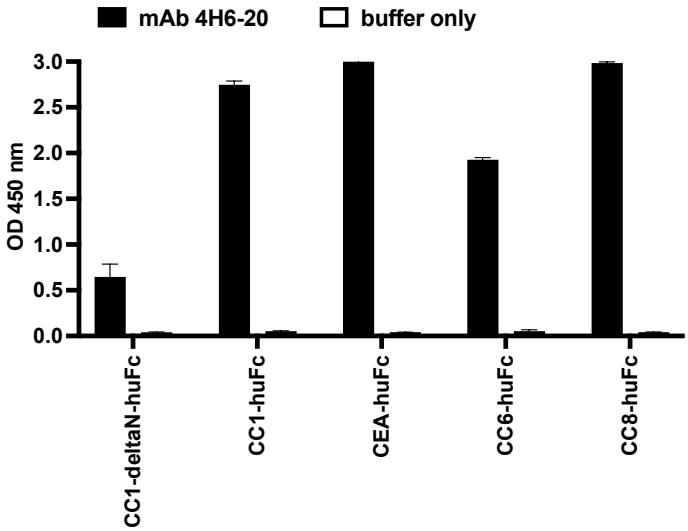
Clone name	4H6-20
Applications tested	Flow Cytometry, ELISA, IF
Clonality & host & isotype	Monoclonal mouse IgG1/kappa
Immunogen	Purified native human CEA
Target molecular weight	CEACAM1: variable; CEACAM5: 77 kDa, apparent 180 kDa comprising 80% carbohydrate; CEACAM6: 90 kDa, CEACAM8: 38 kDa
Recommended dilution	2–10µg/ml
Concentration	mg/ml (lot specific)
Formulation	PBS (pH 7.3), cell culture grade
Purification	purified from cell culture supernatant (ISF-1 Media) by affinity chromatography (protein G)
Storage	Shipped at -20°C or with ice packs, upon delivery store at -20°C. Dilute in PBS (pH7.3) if necessary. Stable for 12 months from date of receipt. Avoid repeated freeze-thaws.
Conjugation	unconjugated
Mouse strain	Balb/c
Fusion partner	P3/NS1/-Ag4.1
Target	**CEACAM1/5/6/8** belong to the 12 members of the **C**arcino**e**mbryonic **a**ntigen receptor CEA related **C**ell **A**dhesion **M**olecules family. **CEACAM1** (CD66a) is the ancestral founder molecule of the CEACAM-family. It is a co-modulator of various receptors, like B-cell, T-cell, EGF-, Insulin-, VEGF-, G-CSR-receptors. CEACAM1 induces B-cell survival and is essential for the resolution of viral infection via protective antiviral antibody production (1,2). **CEACAM5** (**CEA**, CD66e) is expressed in normal inner epithelium of the intestinal tract and can be overexpressed mamma, lung, colorectal and gastrointestinal carcinoma (2). CEA forms homodimers, is highly glycosylated and is membrane associated by a GPI anchor (3). **CEACAM6: (**CD66c), is an approximately 90 kDa GPI-anchored glycoprotein in the CEACAM family. It consists of three immunoglobulin superfamily domains and is membrane associated by a GPI anchor. CEACAM6 is expressed in epithelial cells of the fetal and adult gastrointestinal tract, epithelia of glandular tissues, squamous epithelial cell of the tongue, esophagus and cervix as well as on granulocytes (4). **CEACAM8** is a glycosylphosphatidylinositol (GPI)-linked protein that is solely expressed on granulocytes. It is present in specific vesicles and less on their resting plasma membranes. Upon stimulation, neutrophils secrete a souble form of CEACAM8, that can trigger immunomodulatory activities (4)
UniProt ID	CEACAM1: P13688; CEACAM5 (CEA): P06731; CEACAM6: P40199, CEACAM8: P31997

IMAGE DATA



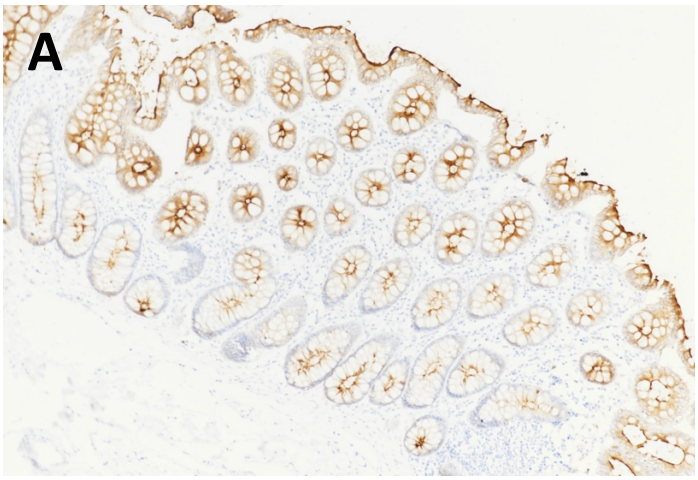
Flow Cytometry analysis of mAb 4H6-20

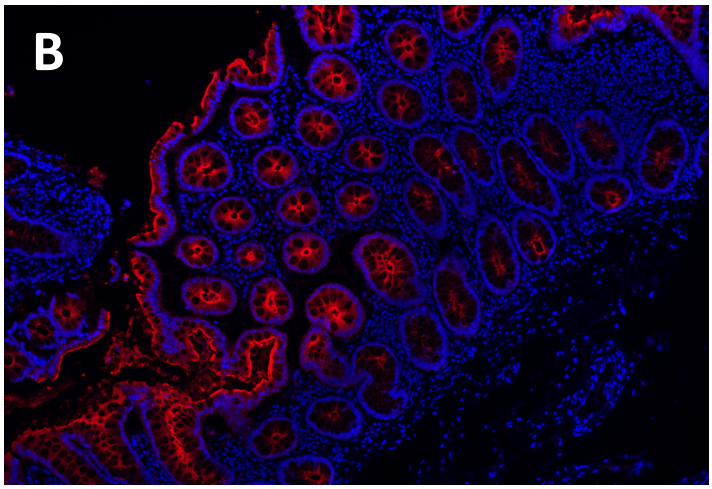
200 000 CHO-cells stably expressing indicated human CEACAMs as well as murine CEACAM1 and macaque CEACAM1 and CEACAM5 (CEA) were stained with the **mAb 4H6-20** (bold line) at 10 µg/ml (66 nM) or detection Ab only (thin line). Detection occurred with a FITC-conjugated anti-mouse IgG.
Binding was observed against CEACAM1-, 5 (CEA)-, 6-, and CEACAM8-transfectants. Cross-reactivity can be also detected against macaque CEACAM5 (CEA).



Indirect Enzyme-Linked Immunosorbent Assay (ELISA):

Indicated CEACAM-huFc antigens were coated onto wells. After blocking with 1% PBS-BSA, mAb **4H6-20** (5 µg/ml) was incubated with the antigens. After washing, HRP-coupled goat anti mouse-IgG was incubated and binding visualized by TMB detection.
MAb **4H6-20** binds to human CEACAM1, 5 (CEA), 6 and CEACAM8.





Immunohistochemistry Analysis
Deparaffinized paraffin-embedded, normal colon-tissue was blocked with 1% BSA/PBS. Sections were incubated overnight at 4°C with the monoclonal antibody clone **4H6-20.**
For bright field detection **(A)**, sections were incubated with biotinylated rabbit anti-mouse secondary Ab (Dako, Germany), followed by treatment with VECTASTAIN ABC reagent (Vector Laboratories, USA) and counterstained with hematoxylin. For immunofluorescence staining **(B)**, incubation occurred with biotinylated rabbit anti-mouse secondary Ab (Dako) followed by Cy-3-(IF) coupled streptavidin (Jackson ImmunoResearch Lab Inc., PA), and counterstained with DAPI.

REFERENCE

(1) Khairnar et al. **CEACAM1 promotes CD8^{+} T cell responses and improves control of a chronic viral infection.** Nat Commun. 2018 Jul 2;9(1):2561. PMID: 29967450

(2) Khairnar et al. **CEACAM1 induces B-cell survival and is essential for protective antiviral antibody production** Nat Commun. 2015 Feb 18:6:6217. PMID: 25692415

(3) Han et al. **The old CEACAMs find their new role in tumor immunotherapy,** Review**,** Invest New Drugs 2020 Dec;38(6):1888-1898. PMID: 32488569

(4) Skubitz Keith **The role of CEACAMs in neutrophil function** *Eur J Clin Invest.* 2024; 54(Suppl. 2):e14349. PMID 39674879