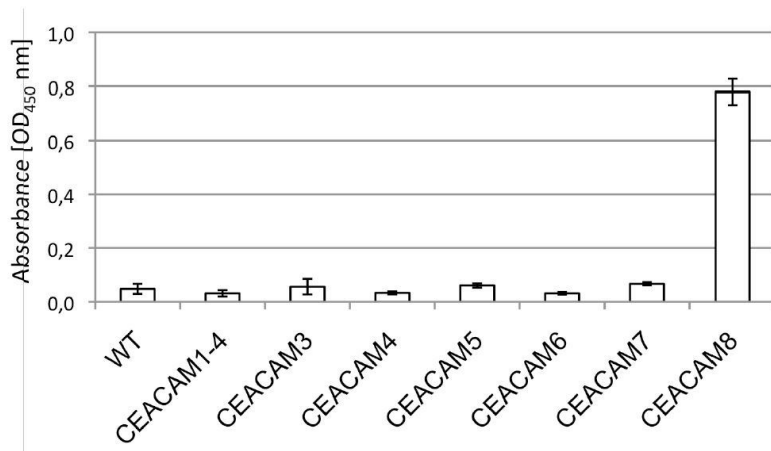


DESCRIPTION:

Clone name	6/40c
Applications	Flow Cytometry, ELISA, IHC, IP, WB
Clonality & host & isotype	Monoclonal mouse IgG1/kappa
Immunogen	pure human CEACAM8-huFc fusion protein (293HEK cells)
Molecular weight of target	Approx. 38 kDa
Recommended dilution	5 – 10 µg/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/NS1/1-Ag4.1
Target	<p>The carcino-embryonic antigen-related cell adhesion molecule 8 (CEACAM8) is a glycosylphosphatidylinositol (GPI)-linked protein also known as CD66b, CD67, CGM6, and NCA-95. CEACAM8 is a member of the carcinoembryonic antigen (CEA)-like, belonging to the immunoglobulin superfamily.</p> <p>CEACAM8, solely expressed on granulocytes, are present in specific vesicles and less present on their resting plasma membranes. Upon stimulation, neutrophils secrete a soluble form of CEACAM8, that can trigger immunomodulatory activities (1).</p> <p>Human granulocytes release and upregulate CEACAM8 in response to bacterial chromatin, thereby negatively regulating Toll-like receptor 2 dependent activation by binding on CEACAM1 on airway epithelial cells (2). Likewise, extracellular chromatin in rheumatoid arthritis- (RA) and systemic lupus erythematosus- (SLE) patients can induce the release of soluble CEACAM8, alternating the immune response by binding to membrane bound CEACAM1 on leukocyte subpopulations (3).</p> <p>Soluble CEACAM8 can act as a co-stimulatory factor on B-cells (4).</p> <p>The monoclonal antibody <b>6/40c</b> directed against CEACAM8 is used to quantify soluble CEACAM8 and to investigate cell signalling-mediated effects on immune responses, including in cancer cells (2,3).</p>
Uniprot ID	P31997

IMAGE DATA



**ELISA of mAb 6/40c:**

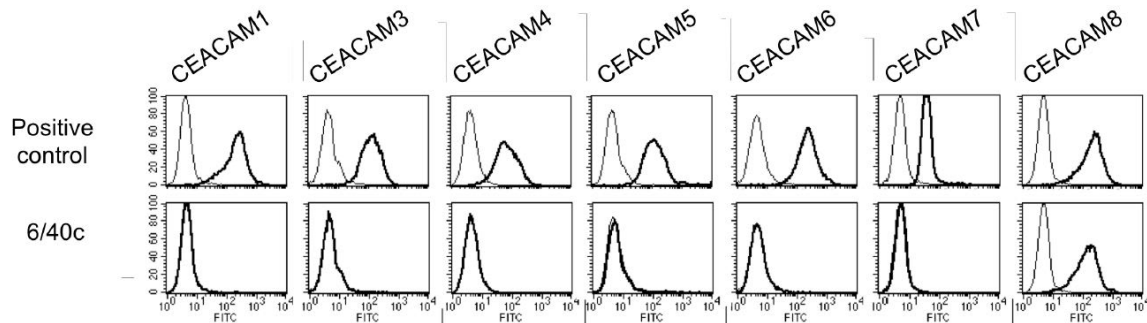
Sandwich ELISA-analysis of CEACAM overexpressing lysates by usage of the mAb **6/40c** as a primary detection antibody.

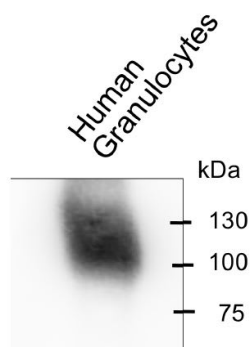
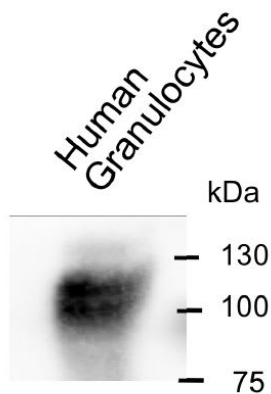
A pan-specific anti human CEACAM was used a catcher-antibody, after which lysates of CHO-cells transfected with indicated CEACAMs were added. Detection of bound CEACAM was performed by using 10 µg/ml of mAb **6/40c** as a primary detection antibody, followed by HRP-coupled goat anti-mouse IgG. TMB was used for visualizing binding and measured by an ELISA reader at 450 nm.

MAb **6/40c** shows strong binding against human CEACAM8, and no binding was detected against human CEACAM1-4, CEACAM3, CEACAM4, CEACAM5 (CEA), CEACAM6 and CEACAM7.

**Flow Cytometry analysis of mAb 6/40c**

250,000 CHO cells transfected with indicated human CEACAM plasmids were stained using 10 µg/ml of a (upper panel) corresponding positive control antibody, or (lower panel) mAb **6/40c**, followed by goat anti mouse FITC. Isotype matched control mAb was used as a negative control (thin line).





### Immunoprecipitation of human CEACAM 8

Human granulocyte lysate was spiked with 10 µg/ml of mAb **6/40c** and immunoprecipitated by protein A Sepharose. Proteins were separated by SDS-PAGE and immunoblotted. Detection of the IP-precipitated proteins occurred by using 5 µg/ml of the pAb Anti-CEA (DAKO, a pan-reactive CEACAM pAb) followed by HRP-coupled secondary IgG.

### Western Blotting Analysis:

Lysate of the human granulocytes was separated by SDS-PAGE and blotted on nitrocellulose membrane. Detection of human CEACAM8 occurred by incubation of mAb **6/40c** (10 µg/ml), followed by HRP-coupled secondary IgG and visualized by Chemiluminescence.

## REFERENCE

- (1) Skubitz Keith **The role of CEACAMs in neutrophil function** *Eur J Clin Invest.* 2024;54(Suppl. 2):e14349. PMC11646290
- (2) Singer BB **Soluble CEACAM8 interacts with CEACAM1 inhibiting TLR2-triggered immune responses.** *PLoS One.* 2014 Apr 17;9(4):e94106. PMC3990526
- (3) Matthieu Ribon, Bernhard B. Singer and Patrice Decker **Extracellular Chromatin Triggers Release of Soluble CEACAM8 Upon Activation of Neutrophils** *Frontiers in Immunology* June 2019 | Volume 10 | Article 1346 published: 14 June 2019, PMC6587075
- (4) Seifert M, Przekopowicz M, Taudien S, et al. **Functional capacities of human IgM memory B cells in early inflammatory responses and secondary germinal center reactions.** *Proc Natl Acad Sci USA.* 2015;112(6):E546-E555. doi:10.1073/pnas.1416276112 PMID: PMC4330750

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