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anti human CEACAM1/3/5
monoclonal antibody
mouse IgG1; clone **SAB135**

DESCRIPTION

Clone name	SAB135
Applications	Flow Cytometry, ELISA, IHC, IP, WB
Clonality & host & isotype	Monoclonal mouse IgG1/kappa
Immunogen	recombinant CEACAM5-huFc produced in HEK293 transfectants
Molecular weight of targets	CEACAM1: variable CEACAM3: 27 kDa CEA: 77 kDa, apparent 180 kDa comprising 80% carbohydrate
Working concentration	2–10 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	**CEACAM1/3/5** 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). **CEACAM3** (CD66d) consists of one IgG like V-domain only, a single transmembrane domain and a cytoplasmatic tail with an immunoreceptor tyrosine-based activation motif (ITAM). It is specifically expressed on human neutrophils and granulocytes, furthermore serves as a ligand for the Opa membrane protein of Neisseria gonorrhoeae (3). **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 (4). The mAb **SAB135** binds to the N-terminal region of the CEACAM1,3 and 5 (5).
UniProt ID	CEACAM1: P13688 CEACAM3: P40198 CEACAM5 (CEA): P06731

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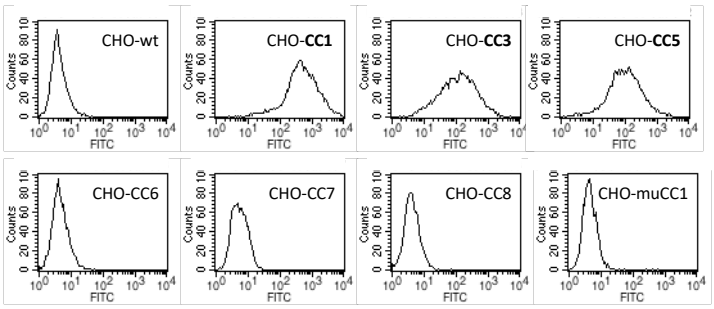
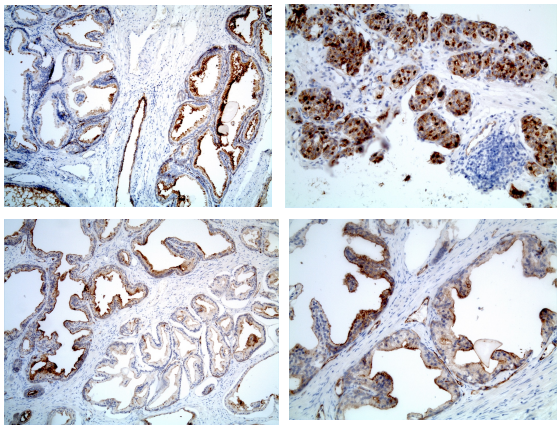
IMAGE DATA



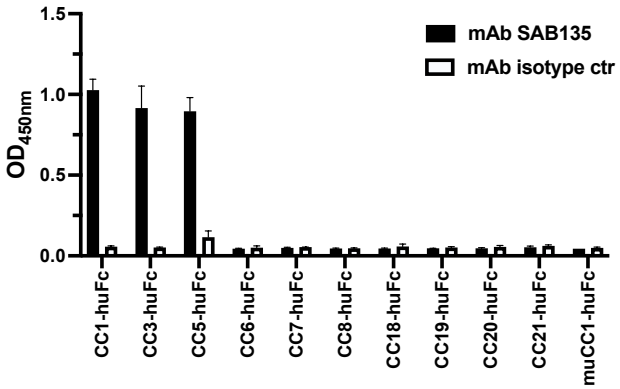
Flow Cytometry analysis

CEACAM transfected CHO cells (or wt) were stained with purified mAb **SAB135**, followed by goat anti mouse FITC and analyzed by flow cytometer. Dead cells were discriminated by staining with propidium iodide (PI) and excluded from the analysis. A shift in FITC-signal could only be detected against CEACAM1,3 and 5, but not against non-transfected CHO-cells or the other CEACAM transfectants.



Immunohistochemistry staining

Formalin fixed prostate tissues were paraffin embedded, deparaffinized and epitope was retrieved by heat induction. Endogenous peroxidase activity was blocked with 3% H_2O_2 and slides blocked with 1 % BSA/PBS. Tissue slides were incubated with 10 µg/ml mAb **SAB135** mAbs in 0.5% BSA/PBS, followed by a biotinylated rabbit anti-mouse Ab and VECTASTAIN ABC reagent (Vector Laboratories, USA). Staining was visualized using diaminobenzidine (DAB) substrate and counterstained with hematoxylin (blue).



Indirect Enzyme-Linked Immunosorbent Assay (ELISA):

SAB135 binds to human CEACAM1/3/5.
Indicated CEACAM-huFc antigens were coated onto wells. After blocking with 2% Milk-PBS, mAb SAB135 (8 µg/ml; black bars) or a murine isotype control mAb (8 µg/ml; white bars) were incubated with the antigens. After washing, HRP-coupled goat anti mouse IgG was incubated and binding visualized by TMB detection.

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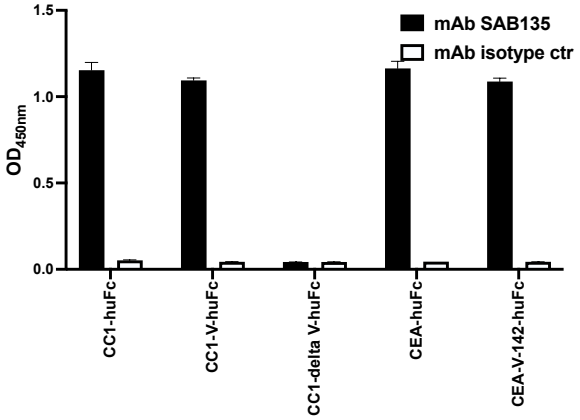
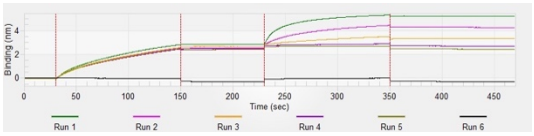


Epitope restriction

The mAb **SAB135** binds to the N-terminal V-domain of human CEACAM1 and CEA. (CEACAM3 consists of one V-domain only) CEACAM1-huFc; CEACAM1-V-huFc (expressing the N terminal V-domain only), CEACAM1 delta V (lacking the N-terminal V-domain; CEA-huFc and CEA-V-142-huFc (expressing the N terminal V-domain only), were coated and assessed for binding against the mAb SAB135 in ELISA.



Immobilized	CEACAM1	CEACAM3	CEACAM5
Kd	43 nM	321 nM	6 nM

Kd determination

The affinity of the mAb **SAB135** against the three recombinant human CEACAM1-huFc, CEACAM3-huFc and CEACAM5-huFc was determined using the BLI-system (fortébio). The highest affinity was observed against CEACAM5 (6 nM), whereas the affinity against CEACAM1 (43nM) and CEACAM3 (321 nM) was respectively 7x and 54x lower.

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) Pils et al**. CEACAM3: an innate immune receptor directed against human-restricted bacterial pathogens** Int J Med Microbiol, 2008 Oct;298(7-8):553-60. PMID: 18606569

(4) 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

(5) Galaski et al. **Fusobacterium nucleatum CbpF Mediates Inhibition of T Cell Function Through CEACAM1 Activation.** Front Cell Infect Microbiol. 2021 Jul 15;11: 692544. PMID: 34336716