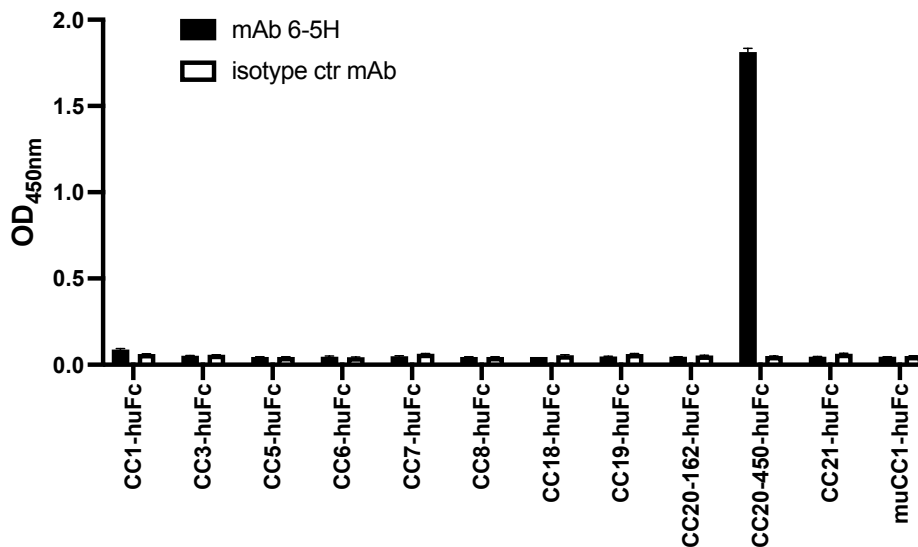


DESCRIPTION

Clone name	6-5H
Applications tested	ELISA, IHC
Clonality & host & isotype	Monoclonal mouse IgG1/kappa
Immunogen	human CEACAM20-huFc
Molecular weight of target	65 kDa, transmembrane anchored glycoprotein, also soluble variants
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	CEACAM20 is a member of the CEACAM gene family with expression limited to the lumen of small intestine, testes, and prostate, is co-expressed with CEACAM1 in adult prostate tissue and down-regulated to the same extent as CEACAM1 in prostate cancer (1). The mature human CEACAM20 consists of a 450 aa in the extracellular domain, a 20 aa helical transmembrane, and a 113 aa cytoplasmic domain (2). The extracellular domain possesses four IgC2like domains with several predicted glycosylation sites, whereas the cytoplasmic domain is unusually long compared to most other CEACAMs and is predicted to contain four tyrosine phosphorylation sites, two of which correspond to the immunoreceptor tyrosine based activation motif (ITAM) (2). Intestinal infection with gram positive bacteria increases the expression of CEACAM20 in the intestinal lining (3).
UniProt ID	Q6UY09

IMAGE DATA

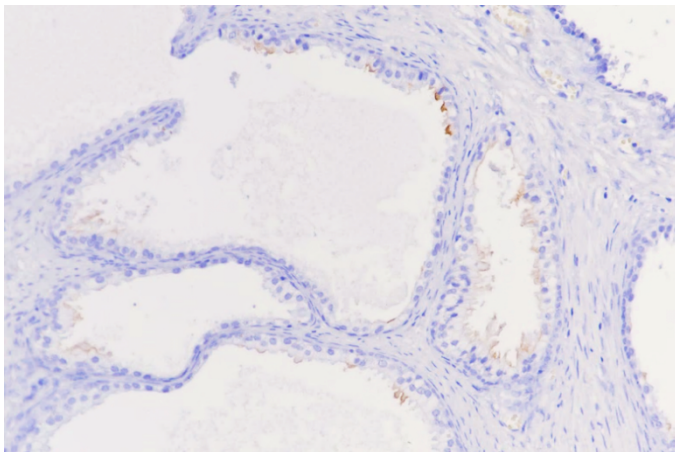


Indirect Enzyme-Linked Immunosorbent Assay (ELISA)

Indicated CEACAM-huFc antigens were coated onto wells. After blocking with 2% Milk-PBS, mAb **6-5H** (8 µg/ml; black bars) or isotype control mAb (white bars) were incubated with the antigens. After washing, HRP-coupled goat anti-mouse antibody was incubated, and bound fraction was visualized by TMB detection. The mAb **6-5H** binds to the CEACAM20 antigen spanning the entire extra cellular domain (until aa 450), however does not bind against the antigen that spans the N-terminal V-domain only (until aa 162), suggesting that mAb **6-5H** binds downstream of the N-terminal domain (aa 163-450).

Immunohistochemistry staining

Formalin fixed human prostate tissue was paraffin embedded, deparaffinized and epitope was retrieved by heat induction. Endogenous peroxidase activity was blocked with 3% H₂O₂ and slides blocked with 1% BSA/PBS. Tissue slides were incubated with 10 µg/ml mAb **6-5H** in 0.5% BSA/PBS, followed by a biotinylated rabbit anti-mouse Ab and VECTASTAIN ABCreagent (Vector Laboratories, USA). Staining was visualized using diaminobenzidine (DAB) substrate and counterstained with hematoxylin (blue). Brown line of staining shows CEACAM20 expression, which appears at the outer layer of inner epithelial cells.



REFERENCE

- (1) Zhang H, Eisenried A, Zimmermann W, Shively JE. **Role of CEACAM1 and CEACAM20 in an in vitro model of prostate morphogenesis.** PLoS One. 2013. PMID: 23358633
- (2) Zebhauser R, Kammerer R, Eisenried A, McLellan A, Moore T, Zimmermann W. **Identification of a novel group of evolutionarily conserved members within the rapidly diverging murine Cea family.** Genomics. 2005 Nov;86(5):566-80. PMID: 16139472
- (3) Kitamura Y, Murata Y, Park JH, Kotani T, Imada S, Saito Y, Okazawa H, Azuma T, Matozaki T. **Regulation by gut commensal bacteria of carcinoembryonic antigen-related cell adhesion molecule expression in the intestinal epithelium.** Genes Cells. 2015 Jul;20(7):578-89. PMID: 25908210

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