## abcam

## Product datasheet

# Alexa Fluor® 488 Anti-beta Catenin antibody [E247] ab194118



Recombinant

RabMAb

## 6 Images

#### Overview

Product name Alexa Fluor® 488 Anti-beta Catenin antibody [E247]

**Description** Alexa Fluor® 488 Rabbit monoclonal [E247] to beta Catenin

Host species Rabbit

Conjugation Alexa Fluor® 488. Ex: 495nm, Em: 519nm

Tested applications Suitable for: Flow Cyt (Intra), ICC/IF

Species reactivity Reacts with: Human

**Immunogen** Synthetic peptide. This information is proprietary to Abcam and/or its suppliers.

Positive control ICC/IF: SW480, Caco-2 and wild-type HAP1 cells. Flow Cyt (Intra): SW480 and wild-type HAP1

cells.

General notes

Our RabMAb® technology is a patented hybridoma-based technology for making rabbit

monoclonal antibodies. For details on our patents, please refer to **RabMAb**® **patents**.

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outlicensing@thermofisher.com.

#### **Properties**

Form Liquid

1

Storage instructions Shipped at 4°C. Store at +4°C short term (1-2 weeks). Upon delivery aliquot. Store at -20°C.

Avoid freeze / thaw cycle. Store In the Dark.

Storage buffer pH: 7.40

Preservative: 0.02% Sodium azide

Constituents: PBS, 30% Glycerol (glycerin, glycerine), 1% BSA

Purity Protein A purified

**Clonality** Monoclonal

Clone number E247
Isotype IgG

#### **Applications**

The Abpromise guarantee Our Abpromise guarantee covers the use of ab194118 in the following tested applications.

The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user.

Application	Abreviews	Notes
Flow Cyt (Intra)		Use a concentration of 0.1 µg/ml.
ICC/IF		1/50 - 1/500.

#### **Target**

**Function** 

Key dowstream component of the canonical Wnt signaling pathway. In the absence of Wnt, forms a complex with AXIN1, AXIN2, APC, CSNK1A1 and GSK3B that promotes phosphorylation on N-terminal Ser and Thr residues and ubiquitination of CTNNB1 via BTRC and its subsequent degradation by the proteasome. In the presence of Wnt ligand, CTNNB1 is not ubiquitinated and accumulates in the nucleus, where it acts as a coactivator for transcription factors of the TCF/LEF family, leading to activate Wnt responsive genes.

Involved in the regulation of cell adhesion. The majority of beta-catenin is localized to the cell membrane and is part of E-cadherin/catenin adhesion complexes which are proposed to couple cadherins to the actin cytoskeleton.

**Tissue specificity** 

Expressed in several hair follicle cell types: basal and peripheral matrix cells, and cells of the outer

and inner root sheaths. Expressed in colon.

Involvement in disease

Defects in CTNNB1 are associated with colorectal cancer (CRC) [MIM:114500].

Note=Activating mutations in CTNNB1 have oncogenic activity resulting in tumor development. Somatic mutations are found in various tumor types, including colon cancers, ovarian and prostate carcinomas, hepatoblastoma (HB), hepatocellular carcinoma (HCC). HBs are malignant

embryonal tumors mainly affecting young children in the first three years of life.

Defects in CTNNB1 are a cause of pilomatrixoma (PTR) [MIM:132600]; a common benign skin

tumor.

Defects in CTNNB1 are a cause of medulloblastoma (MDB) [MIM:155255]. MDB is a malignant, invasive embryonal tumor of the cerebellum with a preferential manifestation in children.

Defects in CTNNB1 are a cause of susceptibility to ovarian cancer (OC) [MIM:167000]. Ovarian cancer common malignancy originating from ovarian tissue. Although many histologic types of ovarian neoplasms have been described, epithelial ovarian carcinoma is the most common form. Ovarian cancers are often asymptomatic and the recognized signs and symptoms, even of late-

stage disease, are vague. Consequently, most patients are diagnosed with advanced disease. Note=A chromosomal aberration involving CTNNB1 is found in salivary gland pleiomorphic adenomas, the most common benign epithelial tumors of the salivary gland. Translocation t(3;8) (p21;q12) with PLAG1.

#### Sequence similarities

Belongs to the beta-catenin family.

Contains 12 ARM repeats.

# Post-translational modifications

Phosphorylation by GSK3B requires prior phosphorylation of Ser-45 by another kinase.

Phosphorylation proceeds then from Thr-41 to Ser-37 and Ser-33.

EGF stimulates tyrosine phosphorylation. Phosphorylation on Tyr-654 decreases CDH1 binding

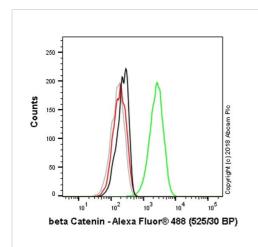
and enhances TBP binding.

Ubiquitinated by the SCF(BTRC) E3 ligase complex when phosphorylated by GSK3B, leading to its degradation. Ubiquitinated by a E3 ubiquitin ligase complex containing UBE2D1, SIAH1, CACYBP/SIP, SKP1, APC and TBL1X, leading to its subsequent proteasomal degradation.

#### **Cellular localization**

Cytoplasm. Nucleus. Cytoplasm > cytoskeleton. Cell junction > adherens junction. Cell junction. Cell membrane. Cytoplasmic when it is unstabilized (high level of phosphorylation) or bound to CDH1. Translocates to the nucleus when it is stabilized (low level of phosphorylation). Interaction with GLIS2 and MUC1 promotes nuclear translocation. Interaction with EMD inhibits nuclear localization.

#### **Images**

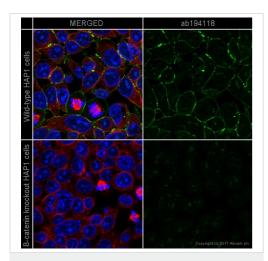


Flow Cytometry (Intracellular) - Alexa Fluor® 488 Anti-beta Catenin antibody [E247] (ab194118) Overlay histogram showing HAP1 wildtype (green line) and HAP1-CNNB1 knockout cells (red line) stained with ab194118. The cells were fixed with 80% methanol (5 min) and then permeabilized with 0.1% PBS-Triton X-100 for 15 min. The cells were then incubated in 1x PBS / 10% normal goat serum to block non-specific protein-protein interactions followed by the antibody (ab194118, 0.1 $\mu$ g/ml) for 30 min at 22°C.

A mouse IgG1 isotype control antibody (ab199091) was used at the same concentration and conditions as the primary antibody (HAP1 wildtype - black line, HAP1-CNNB1 knockout - grey line). Unlabelled sample was also used as a control (this line is not shown for the purpose of simplicity).

Acquisition of >5,000 events were collected using a 50 mW Blue laser (488nm) and 530/30 bandpass filter.

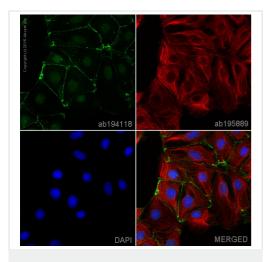
This antibody can also be used in HAP1 cells fixed with 4% formaldehyde (10 min) permeabilized with 0.1% PBS-Triton X-100 for 15 min under the same conditions.



Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-beta Catenin antibody [E247] (ab194118)

ab194118 staining  $\beta$ -catenin in wild-type HAP1 cells (top panel) and  $\beta$ -catenin knockout HAP1 cells (bottom panel). The cells were fixed with 100% methanol (5min), permeabilized with 0.1% Triton X-100 for 5 minutes and then blocked with 1% BSA/10% normal goat serum/0.3M glycine in 0.1% PBS-Tween for 1h. The cells were then incubated with ab194118 at 1/500 dilution (shown in green) and ab195889 at 1/250 dilution (shown in pseudo colour red) overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).

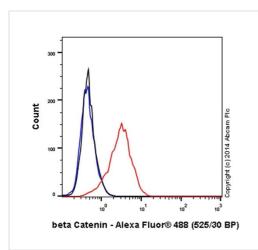


Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-beta Catenin antibody [E247] (ab194118)

ab194118 staining beta-Catenin in Caco-2 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab194118 at a working dilution of 1/100 (shown in green) and <u>ab195889</u>, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at  $2\mu g/ml$  overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product gave a positive signal in 100% methanol (5 min) fixed Caco-2 cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems,  ${\sf TCS\ SP8}$ ).



Flow Cytometry (Intracellular) - Alexa Fluor® 488 Anti-beta Catenin antibody [E247] (ab194118)

ab 194118 ab 195889

DAFI

DAFI

Immunocytochemistry/ Immunofluorescence - Alexa Fluor® 488 Anti-beta Catenin antibody [E247] (ab194118)

Overlay histogram showing SW480 cells stained with ab194118 (red line). The cells were fixed with 4% formaldehyde (10 min) and then permeabilized with 0.1% PBS-Tween for 20 min. The cells were then incubated in 1x PBS / 10% normal goat serum / 0.3M glycine to block non-specific protein-protein interactions followed by the antibody (ab194118, 1/5000 dilution) for 30 min at 22°C. Isotype control antibody (black line) was rabbit IgG (monoclonal) Alexa Fluor® 488 used at the same concentration and conditions as the primary antibody. Unlabelled sample (blue line) was also used as a control.

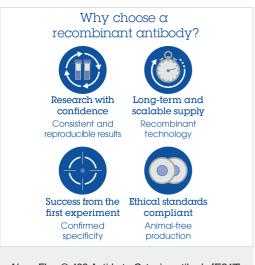
Acquisition of >5,000 events were collected using a 20mW Argon ion laser (488nm) and 525/30 bandpass filter.

This antibody gave a positive signal in SW480 fixed with 80% methanol (5 min)/permeabilized with 0.1% PBS-Tween for 20 min used under the same conditions.

ab194118 staining beta-Catenin in SW480 cells. The cells were fixed with 4% formaldehyde (10 min), permeabilised in 0.1% Triton X-100 for 5 minutes and then blocked in 1% BSA/10% normal goat serum/0.3M glycine in 0.1%PBS-Tween for 1h. The cells were then incubated with ab194118 at a working dilution of 1 in 50 (shown in green) and <u>ab195889</u>, Mouse monoclonal [DM1A] to alpha Tubulin (Alexa Fluor® 594, shown in red) at  $2\mu g/ml$  overnight at +4°C. Nuclear DNA was labelled in blue with DAPI.

This product gave a positive signal in 100% methanol (5 min) fixed SW480 cells under the same testing conditions.

Image was taken with a confocal microscope (Leica-Microsystems, TCS SP8).



Alexa Fluor® 488 Anti-beta Catenin antibody [E247] (ab194118)

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