

## 

# Mouse anti-Claudin- 4-Alexa 488®

### Catalog no. 32-9488

(See product label for lot information)

Clone/PAD: 3F2C1 Isotype: Mouse IqG<sub>1</sub> Qty:  $100 \mu g/200 \mu l$ 

#### **FORM**

This monoclonal antibody is supplied as a 200 µl aliquot at a concentration of 0.5 mg/mL in PBS, pH 7.4, containing 0.1% sodium azide, and BSA to bring the final protein concentration to 4-5 mg/ml after conjugation. Before Conjugation, this antibody is highly purified from mouse ascites by protein A chromatography.

#### **REACTIVITY**

Reactivity has been confirmed with human, rat ileum homogenates mouse MTE 7B and canine MDCK cell lysates by Western blotting (unconjugated) and immunofluorescence. Reactivity has also been confirmed with formalin-fixed, paraffin-embedded (FFPE) human normal colon and colon cancer tissues immunohistochemistry.

#### Validation

See www.invitrogen.com/antibodies for protocols Working concentrations for specific applications should be determined by the investigator. Appropriate concentrations will be affected by experimental conditions. The following concentration ranges are recommended starting points for this product.

Immunofluorescence: 2.5-10 µg/mL 1-3 µg/mL Western Blotting (un-conjugated: Immunohistochemistry: 2-3 µg/mL

#### Immunogen

Synthetic peptide corresponding to a 22 amino acid sequence derived from the C-terminal region of human Claudin-4

#### **SPECIFICITY**

This antibody reacts specifically with the ~ 22 kDa Claudin-4 protein and does not cross-react with the protein at 55 kDa.

#### Storage

2-8°C for up to 1mo, -20°C for long term storage. Avoid repeated freezing and thawing.

#### **Expiration Date**

Expires one year from date of receipt when stored as instructed.

_	<u>Catalog</u> <u>No.</u>	<u>Product</u>	<u>Conjugation</u>	<u>EX</u> (nm)	<u>EM</u> (nm)
	329400	Mouse anti-Claudin-4	Un-conjugated		
	329488	Mouse anti-α-Claudin-4- Alexa488 <sup>®</sup>	Alexa 488 <sup>®</sup>	495	519

#### **BACKGROUND**

Tight junctions are specialized regions of cell-cell contact that are particularly abundant in luminal epithelial cell sheets. In freeze-fracture electron micrographs, tight junctions are visualized as belt-like bands of anastomosing sealing strands (TJ strands) that completely encircle the lateral surfaces of each cell. TJ strands on adjacent cells interact with each other to form a "molecular gasket" that prevents ions, water and other molecules from leaking between cells and thus, from one side of the sheet to the other. In addition to this "barrier" function, the "fence" function of tight junctions plays an important role in maintaining epithelial cell-polarity by blocking the diffusion of membrane proteins between apical (luminal) and basolateral cell surfaces.

Several peripheral membrane proteins are associated with tight junctions including ZO-1, ZO-2, ZO-3, cingulin, the 7H6 antigen, Rab-3b, and symplekin. 1-6 Suggested roles for these proteins include involvement in tight junction assembly and maintenance, signal transduction, and the regulation of tight junction permeability. A growing body of evidence suggests that actin filaments play a major role in regulating tight junction permeability.

Until recently, the only transmembrane protein known to be associated with tight junctions was occludin, an ~65 kDa protein with four transmembrane domains. Despite widespread expectation, a critical structural role for occludin in TJ strands was ruled out by the observation of apparently normal tight junctions formed between cells disrupted at both occludin alleles. A closer examination of isolated tight junctions uncovered two related ~22 kDa, four-transmembrane domain proteins, claudin-1 and claudin-2, with no similarity to occludin. In contrast to occludin, which induces only a small number of short strands at cell-cell contact sites when introduced into fibroblasts lacking tight junctions, claudin-1 and -2 induce networks of strands characteristic of true tight junctions. <sup>8,9</sup> Though inconclusive, these findings suggest that claudin-1 and -2 are major structural components of TJ strands and that occludin plays some other accessory role. Excitement in the tight junction field continues to rise following the recent discovery of claudins -3, -4, -5, -6, -7, and -8 and experiments suggesting that tight junctions in different tissues are comprised of different sets of claudin family proteins.

The overexpression of Claudin-4 was found to decrease paracellular electrical conductance due to a selective decrease in Na+ permeability, with no significant change for Cl-. Claudin-4 is the first to confer ionic selectivity to paracellular transport, leading to the prediction that the combination of different claudins defines the overall selectivity of different junctions. Thus, Claudin-4 forms channels through the tight junctions that discriminate against Na+ ions and are indifferent to Cl- ions.

This product is for research use only. Not for use in diagnostic procedures.

www.invitrogen.com

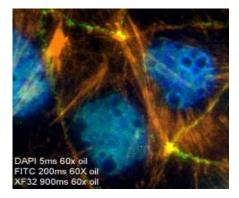
Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com Rev. 0.0



#### **REFERENCES**

- 1. Anderson JM. Curr Biol 6:382-384, 1996.
- Anderson JM, Van Itallie CM. Am J Physiol 269:G467-475, 1995.
- Howarth AG, Stevenson BR. Adv Struct Biol 4:25-39, 1996.
- Stevenson BR, Keon BH. Ann Rev Cell Dev Biol 14:89-109, 1998.
- 5. Tsukita S, et al. Cell Struct Funct 21:381-385,
- 6. Yap AS, et al. Mem Biol 163:159-167, 1998.
- Saitou M, et al. J Cell Biol 141:397-408, 1998.
- 8. Furuse M, et al. *J Cell Biol* 143:391-401, 1998.
- Tsukita S, Furuse M. Genes Cells 3:569-573, 1998.
- 10. Morita K, et al. PNAS 96:511-516, 1999.
- Van Itallie CM, et al. J Clin Invest 107(10):1319-1327, 2001.

### **MOLECULAR PROBES®**



Human Caco-2 cells stained with Mouse anti-α-Claudin-4 – Alexa 488®(Cat.No. 329488) The DNA is counter stained with blue Hoechst 33258 (Cat. No H3569) stain, and actin is stained with Alexa Flour® 568 Phalloidin (Cat. No A12380). For high resolution colored figure, please visit the product page online

This product is for research use only. Not for use in diagnostic procedures.