Qty: 100 µg/200 µl

Mouse anti-Occludin

Catalog No. 33-1500

Lot No. See product label

Mouse anti-Occludin

FORM
This antibody is supplied as a 200 µl aliquot at 0.5 mg/ml in phosphate buffered saline, pH 7.4, containing 0.1% sodium azide. The antibody is Protein A-purified from mouse ascites.

CLONE: OC-3F10

ISOTYPE: Mouse IgG_1-κ.

IMMUNOGEN: GST fusion protein consisting of the C-terminal (150 a.a.) of human occludin fused to GST.

SPECIES REACTIVITY: Human, mouse, rat, canine.

SPECIFICITY
This antibody reacts specifically with mammalian occludin.

REACTIVITY
Reactivity of this antibody with the occludin protein has been confirmed by Western blotting and immunofluorescence. **Tissues/lysates Tested:** T84 cell line (human intestinal epithelium), MDCK cells (canine kidney), Caco-2 cells (human colon adenocarcinoma), and rat liver.

USAGE
The concentrations listed below are good starting points; however, optimal concentrations should be determined by the investigator for each application.

ELISA:	0.1-1.0 µg/ml
Western Blotting:	0.1-1.0 µg/ml
Immunofluorescence:	2-3 µg/ml

Note: For immunofluorescence studies, the following fixation and staining protocol for tissues and cells is recommended- a) incubate sample in ethanol for 30 min at 4 °C, b)following the first incubation, incubate the samples for an additional 3 min. at room temperature with acetone that has been stored at -20, c) block the samples and then incubate with the desired concentration of primary antibody (2-3 µg/ml) overnight at 4 °C, d) incubate with FITC-goat anti-mouse for 1 hour at room temperature.

STORAGE
Store at 2-8°C for up to one month. Store at –20°C for long term storage. Avoid repeated freezing and thawing.

BACKGROUND
The establishment and maintenance of tight junctions is crucial to both the development and normal functioning of most organs[1,2]. These junctions play dual roles in the physiological functions of both epithelial and endothelial cells[1]. Firstly, they function to create a barrier to the diffusion of solutes through the paracellular pathway[1]. Secondly, they function as a boundary between the apical and basolateral plasma membrane domains to create and maintain cell polarity[1]. Tight junctions (TJs) were first observed by electron microscopy over thirty years ago and were defined as a set of continuous, anastomosing intramembrane strands[3]. Yet, information on the molecular organization, assembly, and functional regulation of these junctions has remained scarce. Over the past five years, some progress has been made in the Identification of proteins which constitute TJs. The first TJ protein to be identified was the 220 kDa peripheral membrane protein ZO-1 which

(cont'd)

is localized at TJs in both epithelial and endothelial cells[4,5]. This protein is also expressed in cells which lack TJs such as fibroblasts; however, in these cell types, the ZO-1 protein is localized at adherens junctions[6]. Subsequent studies revealed the existence of a ZO-1 homologue termed ZO-2. ZO-2 is also a peripheral membrane protein, but, unlike ZO-1, ZO-2 is found only at TJs[7]. In addition to ZO-1 and ZO-2, other TJ-specific peripheral membrane proteins have been identified including cingulin, the 7H6 antigen, and symplekin[8,9,10]. Another important discovery was the recent identification of the first transmembrane protein to be localized to tight junctions, termed occludin[11,12,13].

The 65 kDa occludin protein was first identified in chicken using monoclonal antibodies[11,12]. The chicken occludin cDNA was subsequently cloned and sequenced, and the amino acid sequence revealed that the occludin protein is organized into five distinct domains: a short amino terminal cytoplasmic domain (domain A), two extracellular loops (domains B and D) separated by a short intracellular loop (domain C), and a long carboxy-terminal cytoplasmic tail (domain E)[11,12]. The C-terminal tail of occludin is required for both for its localization at tight junctions and for its direct interaction with the ZO-1 protein[12]. One interesting feature of the occludin protein is that its amino acid sequence has not been highly conserved throughout evolution[13]. This fact made isolating the mammalian homologues of chicken occludin a rather difficult task. Recently, however, the sequences of the full length cDNAs encoding occludin of rat-kangaroo, human, mouse, and dog were reported[13]. At the amino acid level, the human, murine, and canine occludin proteins are highly homologous (~ 90% identity); however, the mammalian proteins exhibit a considerable degree of divergence from the rat-kangaroo and chicken proteins[13]. Nevertheless, the overall structural features of the occludin protein are highly conserved in all the species examined[13]. The recent identification and cloning of the mammalian occludin protein will undoubtedly facilitate the further study of TJ organization and function.

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RELATED PRODUCTS

Product	***Clone***	***Cat. No***
Rb anti-Claudin	MH25	71-7800
Rabbit anti-Occludin	Z-T22	71-1500
Rabbit anti-ZO-1	Z-R1	61-7300
Rabbit anti-ZO-2	Polyclonal	71-1400
Mouse anti-E-Cadherin	HECD-1	13-1700
Rabbit anti-α-Catenin	ZER2	71-1200
Mouse anti-β-Catenin	CAT-5H10	13-8400
Mouse anti-γ -Catenin	PG-11E4	13-8500

Product	***Conjugate***	***Cat. No.***
Goat anti-Mouse IgG (H+L) (ZyMAX™ Grade)	Purified	81-6500
	FITC	81-6511
	TRITC	81-6514
	Cy™3	81-6515
	Cy™5	81-6516
	HRP	81-6520
	AP	81-6522
	Biotin	81-6540
Protein A	Sepharose® 4B	10-1041
rec-Protein G	Sepharose® 4B	10-1241

(Unlike the Rabbit anti-Occludin polyclonal antibody (71-1500), the mouse monoclonal antibody does not appear to detect the most highly phosphorylated form of the occludin protein when tested on Caco-2 cells. Therefore, the OC-3F10 antibody may have more limited utility than the polyclonal antibody for detecting the state of occludin phosphorylation under different conditions.)

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