Qty: 100 µg/400 µl

Rabbit anti-Occludin

Catalog No. 71-1500

Lot No. See product label

Rabbit anti-Occludin

FORM
This polyclonal antibody is highly purified from rabbit antiserum by epitope affinity chromatography using a fusion protein-Occludin coupled gel. The antibody is supplied as a 400 µl aliquot at a concentration of 0.25 mg/ml in PBS, pH 7.4, containing 0.1% NaN_3.

IMMUNOGEN
A fusion protein containing the C-terminal 150 amino acid region of human occludin.

SPECIFICITY
This antibody reacts specifically with mammalian occludin including human, mouse, rat, and dog occludin. On Western blots, it identifies the target band at ~65 kDa and since this protein is post-translationally modified by hyperphosphorylation, higher molecular weight species ranging from 65 to 79 kDa have been observed.

REACTIVITY
Reactivity of this antibody with the occludin protein has been confirmed by Western blotting using total cell lysates derived from MDCK cells, human T84 cells, human Caco-2, and rat fibroblasts transfected with a plasmid encoding human occludin.

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

ELISA:	0.1 - 1.0 µg/ml
Western Blotting[15,20]:	1 - 3 µg/ml
Immunofluorescence[15-18]:	4 - 20 µg/ml
Immunoprecipitation[19]:	~5 µg/IP reaction
Immunohistochemistry (FFPE):	2-5 µg/ml

For immunofluorescence studies, the following fixation and staining protocol is recommended- a) "pre-extract" the cells on ice for 2 min. with an ice-cold buffer containing 0.2% Triton X-100[14]; b) Fix cells in 3% paraformaldehyde in PBS for 30 min at room temperature; c) permeabilize sample with 0.05% TX-100 in PBS for 5 min on ice; c) incubate samples with the desired concentration of primary antibody (4-20 µg/ml) on ice for 1 hour; d) incubate with FITC-goat anti-rabbit for 0.5 hour at room temperature.

Important: *To achieve appropriate staining results on formalin-fixed, paraffin-embedded tissue sections (FFPE), protease pre-treatment is required (Sigma #P-5147). The recommended dilution and incubation for protease is 1-2 mg/ml at 37° C for 10 minutes. Other enzyme and HIER (Heat Induced Epitope Retrieval) pre-treatment methods may not be suitable for this particular antibody.*

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 epithelia.[1,2] These junctions play dual roles in the physiological functions of both epithelial and endothelial cells.[1] First, they function to create a barrier to the diffusion of solutes through the paracellular pathway.[1] Second, 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 is localized at TJs in both epithelial and endothelial cells.[4,5] This protein is also expressed in cells which lack TJ 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 TJ.[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 through 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 to the 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/PAD**	*Cat. No.*
Claudin-1	MH25	71-7800
Claudin-2	MH44	51-6100
Ms x ZO-1	ZO1-1A12	33-9100
Ms x ZO-1-FITC	ZO1-1A12	33-9111
Rb x ZO-1	Z-R1	61-7300
Rb x ZO-2	--	71-1400
Ms x Occludin	OC-3F10	33-1500
Ms x Occludin-HRP	OC-3F10	33-1511
Ms x Occludin-FITC	OC-3F10	33-1520
Rb x Occludin	Z-T22	71-1500
Rb x Occludin	--	71-1600

*PAD- polyclonal antibody designation

Conjugate	ZyMAX™ Goat x Rabbit IgG (H+L)	ZyMAX™ Goat x Mouse IgG (H+L)
Purified	81-6100	81-6500
FITC	81-6111	81-6511
TRITC	81-6114	81-6514
Cy™3	81-6115	81-6515
Cy™5	81-6116	81-6516
HRP	81-6120	81-6520
AP	81-6122	81-6522
Biotin	81-6140	81-6540