**Qty:** 100 µg

Mouse anti-Occludin-
FITC conjugate

**Catalog No.** 33-1511

**Lot No.** **See product label**

# Mouse anti-Occludin-FITC

## FORM
This antibody is supplied as a 200 µl aliquot at an antibody concentration of 0.5 mg/ml in 50% glycerol, phosphate buffered saline (pH 7.4), 1% BSA containing 0.1% sodium azide as a preservative. The antibody is Protein A-purified from mouse ascites before conjugation to fluoroscein isothiocyanate.

**CLONE:** OC-3F10 **ISOTYPE:** Mouse $IgG_1$-κ **FLUOROCHROME/ANTIBODY RATIO:**

**IMMUNOGEN:** Fusion protein containing the C-terminal ~150 amino acids of human occludin.

## SPECIFICITY
This anybody is specific for the ~65 kDa Occludin protein. In contrast to Zymed's polyclonal rabbit anti-Occludin antibody (cat. no. 71-1500), this antibody does not appear to detect the most highly phosphorylated form of occludin in Caco-2 cells.

## REACTIVITY
This antibody reacts with human, mouse, rat, and dog occludin. Reactivity has been confirmed by Western blotting and by immunofluorescence using T84 cell line (human intestinal epithelium), MDCK cells (canine kidney), Caco-2 cells (human colon adenocarcinoma), and rat liver.

## USAGE
Working concentrations for specific applications should be determined by the investigator. Appropriate concentrations will be affected by several factors, including secondary antibody affinity, antigen concentration, sensitivity of detection method, temperature and length of incubations, etc. We recommend the following ranges as starting points for this product.

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| **ELISA:** | 0.1-1.0 µg/ml |
| **Immunofluorescence:** | 2-10 µg/ml |

Note: For immunofluorescence studies with tissue or cells, Zymed suggests the following fixation and staining protocol: 1) incubate sample in ethanol for 30 min at 4 °C, 2) following ethanol treatment, incubate samples for 3 min. at room temperature with cold (-20° C) acetone, 3) block samples with a blocking buffer, 4) incubate with anti-Occludin-FITC overnight at 4° C.

## STORAGE
Store at 2-8°C.

## 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 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 is found only at TJs[7]. In addition to ZO-1 and ZO-2, other TJ-specific peripheral membrane proteins have been identified

(cont'd)

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.

## REFERENCES


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## RELATED PRODUCTS

| *Product* | *Clone/PAD** | *Cat. No.* |
|---|---|---|
| Rb x Occludin | Z-T22 | 71-1500 |
| Rb x ZO-1 | Z-R1 | 61-7300 |
| Ms x anti-ZO-1 | ZO-1-1A12 | 33-9100 |
| Ms x anti-ZO-1-FITC | ZO-1-1A12 | 33-9111 |
| Rb x Claudin-1 | MH25 | 71-7800 |
| Rb x Claudin-2 | MH25 | 51-6100 |
| Rb x anti-ZO-2 | Polyclonal | 71-1400 |
| Ms x E-Cadherin | HECD-1 | 13-1700 |
| Rb x anti-α-Catenin | ZER2 | 71-1200 |
| Ms x β-Catenin | CAT-5H10 | 13-8400 |
| Ms x γ -Catenin | PG-11E4 | 13-8500 |

*PAD, polyclonal antibody designation

| | | |
|---|---|---|
| Protein A | Sepharose® 4B | 10-1041 |
| rec-Protein G | Sepharose® 4B | 10-1241 |

| 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 |



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