Rabbit anti-ZO-1

For Research Use Only
Lot No.

[X] 18-7430 0.5 mL Concentrate Antibody

INTENDED USE

For research use only. Not for use in diagnostic procedures.

Invitrogen's polyclonal Rabbit anti-ZO-1 antibody is intended to qualitatively stain ZO-1 in frozen and formalin-fixed, paraffin-embedded tissue sections.

SPECIFICITY AND REACTIVITY

ZO-1 and occludin normally localize to the apical aspect of breast epithelial cells. Expression of ZO-1 is associated with gland formation in breast cancer and has been reported in low-grade invasive ductal carcinomas[1].

In a recent study[2], ZO-1 and Occludin expression was completely lost in the invasive component of invasive lobular breast carcinoma. It is possible that loss of cellular polarity as indicated by lack of expression of tight junction proteins ZO-1 and Occludin may be an early event in lobular breast carcinoma, and thus, these two proteins may be possible markers for lobular carcinoma.

Low expression levels of claudin-1 and ZO-1 have been associated with higher tumor grade in colon cancer[3]. Cytoplasmic staining of ZO-1 has been observed in high grade pancreatic intraepithelial neoplasia, suggesting that ZO-1 may be helpful in the identification of high grade lesions[4].

REAGENT PROVIDED

Rabbit anti-ZO-1 is purified from rabbit antisera and diluted in phosphate buffered saline (PBS), pH 7.4, and 1% bovine serum albumin (BSA) with 0.1% sodium azide (NaN_3) as a preservative.

Immunogen: Peptide sequence from the N-terminal region of human ZO-1

Total protein concentration: g/L

PAD: ZMD.437

Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Pancreas, prostate or small intestine

EXPECTED STAINING PATTERN: Membrane, may display cytoplasmic staining in tumor cells.

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

Epitope Retrieval: Not required (See page 2 for protocol)
Enzyme Digestion: Required (Digest-All™ 3) (See page 2 for protocol)

Rabbit anti-ZO-1 may be diluted according to Table 1 when using the Invitrogen detection systems below.

Table 1. Dilution Table

Invitrogen Kit	Predilute Antibody	Dilution for Concentrate	Incubation Time
Histostain-SP or SAP kits*	Ready-To-Use	1: 50 - 1: 100	60 min.
Histostain®-*Plus* Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
SuperPicTure™ Polymer Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
Cap-*Plus*™ Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.

*** Use Histostain-SP or -SAP kits only for Cat. No. 08-0XXX and 18-X001 to 18-X200 primary antibodies.**

This is a guideline only. Optimal antibody concentrations may vary based on specimen and preparation method used, and should be determined by each individual laboratory.

SPECIMEN PREPARATION

1. Use tissue fixed in 10% neutral buffered formalin or other fixative on regular basis, or frozen tissue sections.
2. Cut 3-4 µm sections and place on positively charged slides.
3. Dry overnight at 37° C or for 2-4 hrs at 58°C.

PRETREATMENT

Heat Induced Epitope Retrieval (HIER), if required

1. Deparaffinize slides. Tissue sections should be mounted on silane, poly-L-Lysine, or HistoGrip (Cat. No. 00-8050) coated slides.
2. Wash slides with distilled water 3 times for 2 min each.
3. Place a 1L glass (Pyrex) beaker containing 500 ml of 0.01 M citrate buffer (Cat. No. 00-5000) or EDTA solution (Cat. No. 00-5500) on a hot plate. Heat the buffer solution until it boils. *(This step may be prepared before slide deparaffinization, as the buffer may take several minutes to boil).*
4. Put the slides in a slide rack and place in the beaker with boiling buffer. Keep it boiling for 15 minutes.
5. After heating, remove beaker from the hot plate and allow it to cool down for at least 15-20 minutes at room temperature.
6. Rinse slides with PBS (Cat. No. 00-3000) and begin the immunostaining protocol.

Enzyme Digestion, if required

1. Prewarm the enzyme of choice at 37ºC for 10 min.
2. Add the prewarmed enzyme to a tissue section and incubate at 37ºC for 10 min.
3. Wash in several changes of PBS (Cat. No. 00-3000) and begin the immunostaining protocol.

RECOMMENDED MANUAL STAINING PROCEDURE

1. Submerge slides in peroxidase quenching solution and rinse with PBS.
2. Apply serum blocking solution.
3. Apply primary antibody and incubate for 30-60 min at room temperature; rinse with PBS.
4. Apply secondary antibody and incubate for 10 min at room temperature; rinse with PBS.
5. Apply enzyme conjugate and incubate for 10 min at room temperature; rinse with PBS.
6. Apply chromogen and incubate for 5-10 min at room temperature; rinse with PBS.

MATERIALS REQUIRED BUT NOT PROVIDED

	Reagent	Catalog No.
1.	HistoGrip™	00-8050
2.	Super PAP Pen	00-8899
3.	Isotype Control for Rabbit or Mouse Primary Antibody	08-6199 or 08-6599
4.	Antibody Diluent	00-3118
5.	PBS (0.01 M PBS)	00-3000
6.	Mayer's Hematoxylin	00-8011
7.	Citrate Buffer pH 6.0 (**if required for HIER**)	00-5000
8.	EDTA Solution (**if required for HIER**)	00-5500
9.	Digest-All™ 1, Digest-All™ 2, or Digest-All™ 3 (**if required for Enzyme Digestion)**	00-3007 or 00-3008 or 00-3009
10.	*SuperPicTure*™ polymer kit, or LAB-SA kit (Histostain®-*Plus*, and Cap-*Plus*™).	
11.	Chromogen/substrate (if not included in detection kit): *Single Solution* AEC (Cat. No. 00-1111), or DAB (Cat. No. 00-2014), or Fast-Red (Cat. No. 00-2234).	
12.	Mounting solution: Histomount™ (for DAB: Cat. No.00-8030), GVA (for AEC, or Fast-Red: Cat. No. 00-8000), or Clearmount™ (for AEC, DAB, or Fast-Red: Cat. No. 00-8010).	

REFERENCES

1. Hoover KB, et al. *Am J Pathol* 153(6):1767-73, 1998.
2. Agarwal B, et al. USCAP 2005, Abstract #94.
3. Lee SK, et al. *Oncol Rep* 13(2):193-9, 2005.
4. Cibull T, et al. USCAP 2005, Abstract #1284.

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