Rabbit anti-Smad3
For Research Use Only
Lot No.

[X] 18-7396    0.5 mL Concentrate Antibody

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

Invitrogen’s polyclonal Rabbit anti-Smad3 antibody is intended to qualitatively stain cells in frozen and formalin-fixed, paraffin-embedded tissue sections.

SPECIFICITY AND REACTIVITY
Smad proteins mediate signaling events leading to transcriptional activation initiated by members of the TGF-β superfamily. Biological activities mediated by smads include cell growth and morphogenesis, development, and immune response. A reduction in Smad2/3 signaling enhances tumorigenesis, but also suppresses metastasis of breast cancer cell lines.1 Loss of Smad3 expression also increase susceptibility to tumorigenicity in human gastric cancer. Suppression of Smad3 expression may mediate key oncogenic properties of TGF-β.2 The down regulation of Smad3 was also observed in high-grade breast cancer.3

Invitrogen’s Rabbit anti-Smad3 showed moderate to strong positive nuclear staining on 9/14 breast cancers, and weak positive nuclear staining on 3/3 colon cancers. It also showed positive staining in basal cell carcinoma of the skin, malignant fibrous histocytoma, synovial sarcoma, round cell liposarcoma, leiomyosarcoma, adrenal adenoma, Schwannoma, and ganglioeuroma.

REAGENT PROVIDED
Rabbit anti-Smad3 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 (NaN₃) as a preservative.

Immunogen: Synthetic peptide derived from a central portion of the linker domain of human Smad3

PAD: LPC3
Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Breast cancer

EXPECTED STAINING PATTERN: Mainly nuclear. Weak to moderate cytoplasmic staining was observed in some normal tissues.

INSTRUCTIONS FOR USE
PRETREATMENT REQUIREMENTS:
Epitope Retrieval: Required (EDTA, pH 8.0) (See page 2 for protocol)
Enzyme Digestion: Not Required

Rabbit anti-Smad3 may be diluted according to Table 1 when using the Invitrogen detection systems below.

Table 1. Dilution Table

<table>
<thead>
<tr>
<th>Invitrogen Kit</th>
<th>Predilute Antibody</th>
<th>Dilution for Concentrate</th>
<th>Incubation Time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histostain-SP or SAP kits*</td>
<td>Ready-To-Use</td>
<td>1: 50 - 1: 100</td>
<td>60 min.</td>
</tr>
<tr>
<td>Histostain®-Plus Kits</td>
<td>Ready-To-Use</td>
<td>1: 100 - 1: 200</td>
<td>30-60 min.</td>
</tr>
<tr>
<td>SuperPicTure™ Polymer Kits</td>
<td>Ready-To-Use</td>
<td>1: 100 - 1: 200</td>
<td>30-60 min.</td>
</tr>
<tr>
<td>Cap-Plus™ Kits</td>
<td>Ready-To-Use</td>
<td>1: 100 - 1: 200</td>
<td>30-60 min.</td>
</tr>
</tbody>
</table>

* 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 37°C for 10 min.
2. Add the prewarmed enzyme to a tissue section and incubate at 37°C 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

<table>
<thead>
<tr>
<th>Reagent</th>
<th>Catalog No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. HistoGrip™</td>
<td>00-8050</td>
</tr>
<tr>
<td>2. Super PAP Pen</td>
<td>00-8899</td>
</tr>
<tr>
<td>3. Isotype Control for Rabbit or Mouse Primary Antibody</td>
<td>08-6199 or 08-6599</td>
</tr>
<tr>
<td>4. Antibody Diluent</td>
<td>00-3118</td>
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<tr>
<td>5. PBS (0.01 M PBS)</td>
<td>00-3000</td>
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<tr>
<td>6. Mayer’s Hematoxylin</td>
<td>00-8011</td>
</tr>
<tr>
<td>7. Citrate Buffer pH 6.0 (if required for HIER)</td>
<td>00-5000</td>
</tr>
<tr>
<td>8. EDTA Solution (if required for HIER)</td>
<td>00-5500</td>
</tr>
<tr>
<td>9. Digest-All™ 1, Digest-All™ 2, or Digest-All™ 3 (if required for Enzyme Digestion)</td>
<td>00-3007 or 00-3008 or 00-3009</td>
</tr>
<tr>
<td>10. SuperPicTure™ polymeric kit, or LAB-SA kit (Histostain®-Plus, Histostain®ST5050™, and Cap-Plus™) (see Table 2).</td>
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<tr>
<td>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).</td>
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<tr>
<td>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).</td>
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</table>

REFERENCES

TRADEMARKS
Cap-Plus™, Clearmount™, Digest-All™, HistoGrip™, Histomount™, and Histostain®, PicTure™, and Zyomed® are trademarks of Invitrogen Corporation.

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