



Rabbit anti-STEAP

For Research Use Only

Lot No.

[] 18-7385 0.5 mL Concentrate Antibody
[X] 08-1385 6.0 mL 2nd Gen Predilute Antibody, Ready-To-Use

INTENDED USE

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

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

SPECIFICITY AND REACTIVITY

STEAP (Six Transmembrane Epithelial Antigen of the Prostate) is a novel gene that is highly expressed in advanced prostate cancer. STEAP encodes a 36 kDa protein with six potential membrane-spanning regions flanked by hydrophilic amino- and carboxyl-terminal domains.¹ STEAP has been shown to be predominantly expressed in human prostate tissue, and is up-regulated in multiple cancer cell lines including prostate, bladder, colon, ovarian, and Ewing's sarcoma.¹

STEAP is different from the currently known prostate cancer markers because of its putative secondary structure, from which one may predict that it functions as a potential channel or transporter protein.

Invitrogen's Rabbit anti-STEAP showed moderate to strong positive membrane and cytoplasmic staining on 7/7 prostate cancer, and weak positive staining on 4/4 normal prostate gland. It also showed positive staining on pancreas cancer, thymic carcinoid tumor paraganglioma and ovarian cancer tissues.

On Western blots, Rabbit anti-STEAP identifies a band at ~36kDa in human LNCap-FGC prostate carcinoma cell lysates.

REAGENT PROVIDED

Rabbit anti-STEAP 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: N-terminus of human STEAP

Total protein concentration: g/L

Clone/PAD: ZMD.265

Isotype: IgG

Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Prostate cancer tissue

EXPECTED STAINING PATTERN: Membranous and cytoplasmic

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

Epitope Retrieval: Required (Citrate Buffer, pH 6.0) (See page 2 for protocol)
Enzyme Digestion: Not Required

Rabbit anti-STEAP 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.
Non-Biotin Amplification (NBA) [™] Kits	Ready-To-Use	1: 100 - 1: 200	30-60 min.
PicTure [™] -Plus 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.

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Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com

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(Rev 08/08) DCC-08-1089

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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

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. PicTure™-Plus polymer kit, NBA™ kit, or LAB-SA kit (Histostain®-Plus, HistoST5050™, and Cap-Plus™) (see Table 2).	
11. Chromogen/substrate (if not included in detection kit): <i>Single Solution</i> 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).	

Table 2. Invitrogen Immunohistochemistry Detection Kits

Detection Kits	Enzyme	Chromogen	Size	1° Ab Reactivity	Invitrogen Cat. No.
Histostain [®] -Plus	HRP	AEC	15 mL	Broad Spectrum*	85-9943
Histostain [®] -Plus	HRP	AEC	15 mL	Mouse/Rabbit	85-6543/ 85-6143
Histostain [®] -Plus	HRP	DAB	15 mL	Broad Spectrum*	85-9643
Histostain [®] -Plus	HRP	DAB	15 mL	Mouse/Rabbit	85-9143/ 85-9243
Histostain [®] -Plus	AP	Fast-Red	15 mL	Broad Spectrum*	85-9942
Histostain [®] -Plus	AP	**	60 mL	Broad Spectrum*	85-8942
Histostain [®] -Plus	HRP	**	110 mL	Mouse	85-6643
Histostain [®] -Plus	HRP	**	110 mL	Rabbit	85-6743
Histostain [®] -Plus	HRP	**	110 mL	Broad Spectrum*	85-9043
Picture [™] -Plus Polymer Kit	HRP	AEC	15 mL	Broad Spectrum*	87-9963
Picture [™] -Plus Polymer Kit	HRP	DAB	15 mL	Broad Spectrum*	87-9663
Picture [™] -Plus Polymer Kit	HRP	DAB	15 mL	Mouse/Rabbit	87-9163/ 87-9263
Picture [™] -Plus Polymer Kit	HRP	**	110 mL	Broad Spectrum*	87-8963
Cap-Plus [™] Detection Kit	HRP	DAB	110 mL	Broad Spectrum*	87-8143
Cap- Plus [™] Buffer Kit	For use with Cap-Plus [™] Detection Kit				87-0003
NBA [™]	HRP	AEC	15 mL	Broad Spectrum*	85-3043
NBA [™]	HRP	DAB	15 mL	Broad Spectrum*	85-4043
NBA [™]	HRP	**	60 mL	Broad Spectrum*	85-3243
NBA [™]	HRP	**	110 mL	Broad Spectrum*	85-3943

*Detects mouse, rabbit, rat, and guinea pig primary antibodies

** Bulk kit does not contain Chromogen/Substrate

RELATED PRODUCTS

Table 3. Related Products

Specificity	Clone/PAD	Catalog Number
Mouse anti-PSA	Z009	18-0044
Mouse anti-PSA	Z009	08-1044
Rabbit anti-PSMA	ZMD.80	18-7318
Rabbit anti-PsAP	Polyclonal	08-0045
Rabbit anti-PTEN	PN37	18-0256
Rabbit anti-APO-D	8CD6	18-7253
Mouse anti-Androgen Receptor	AR27	08-1292

REFERENCES

1. Hubert RS, et al. Proc Natl Acad Sci USA 96(25):14523-14528, 1999.

TRADEMARKS

Cap-Plus[™], Clearmount[™], Digest-All[™], HistoGrip[™], Histomount[™], and Histostain[®], NBA[™], PicTure[™], and Zymed[®] are trademarks of Zymed Laboratories, Inc.

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