

Mouse anti-CD44v6, Clone 2F10

For Research Use Only

Lot No.

- [X] 18-7295 0.5 mL Concentrate Antibody
 [] 08-1295 6.0 mL 2nd Gen Predilute Antibody, Ready-To-Use

INTENDED USE

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

Invitrogen's monoclonal Mouse anti-CD44v6 antibody is intended to qualitatively stain the CD44v6 antigen in frozen and formalin-fixed, paraffin-embedded tissue sections.

SPECIFICITY AND REACTIVITY

CD44 (H-CAM, Homing Cell Adhesion Molecule) is a 85-90 kDa transmembrane protein. The human CD44 gene contains 19 exons; 1-5 and 15-19 are expressed by wild-type CD44, also known as the standard hemopoietic form (CD44s). CD44 variants (CD44v) express the same exons as wild-type CD44, with the additional expression of exons in the middle region. Recent studies have shown that expression of CD44 on the cell surface changes profoundly during tumor metastasis.

In the last decade, CD44s and variant isoforms have been used widely in IHC with different tumors. It has been reported that CD44v3 and CD44v6 (containing additional exons 7 and 10, respectively) are associated with tumor progression and metastasis in many types of tumors, including breast, colon, lung, renal, skin, and ovarian cancers. However, staining results correlated with different prognoses in different tumor types. For example, well-differentiated squamous carcinoma demonstrated strong CD44v6 positive reactivity, with reduced expression correlated with invasion and poor prognosis.¹⁻³ This expression profile was also consistent in colon adenocarcinoma,⁴ endocrine pancreatic tumors,⁵ pulmonary adenocarcinoma,⁶⁻⁸ endometrial cancer,⁹ and soft tissue sarcomas.¹⁰ In contrast, in liver hepatocellular carcinoma,¹¹ thyroid papillary adenocarcinoma,¹² gastric carcinoma,¹³ and some breast cancers,¹⁴ high levels of CD44v6 expression together with low levels of nm23-H1 expression may predict higher risk of metastasis and shorter survival time.

REAGENT PROVIDED

Mouse anti-CD44v6 is purified from mouse ascites 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: Human chimeric fusion protein

Total protein concentration: g/L

Clone: 2F10

Isotype: Mouse IgG_{2b}

Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Breast carcinoma

EXPECTED STAINING PATTERN: Mainly membrane

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

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

Mouse anti-CD44v6 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.

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, 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).	

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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-CD44 (H-CAM) (Predilute)	DF1485	08-0184
Mouse anti-CD44v3 (Concentrate)	2F10	18-7294
Mouse anti-CD44v3 (Predilute)	2F10	08-1294

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TRADEMARKS

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