Mouse anti-BAG-1, Clone 10B6H7

For Research Use Only
Lot No.

[X] 18-7481 0.5 mL Concentrate Antibody

INTENDED USE

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

Invitrogen's monoclonal Mouse anti-BAG-1 antibody is intended to qualitatively stain BAG-1 in frozen and formalin-fixed, paraffin-embedded tissue sections.

SPECIFICITY AND REACTIVITY

BCL2-associated athanogene 1 (BAG-1) is a multifunctional protein that interacts with Bcl-2, HSP 70 and a wide range of target molecules to block apoptosis, regulate proliferation, transcription, metastasis and motility. BAG-1 immunoreactivity is detected in a wide variety of cell types in normal adult tissues and is localized to either cytosol, nucleus, or both, depending on the cell.[1]

Pilot clinical studies have demonstrated that overexpression of nuclear BAG-1 could be associated with shorter survival periods in patients with breast and laryngeal carcinomas.[2] Overexpression of cytoplasmic BAG-1, however, may be associated with a better clinical outcome in early stage breast cancer and in non-small cell lung cancer.[2] BAG-1 expression is frequently altered in a range of human cancers relative to normal cells.[3] The expression of BAG-1 correlates with that of Bcl-2, p53, differentiation, estrogen and progesterone receptors in breast cancer.[4] BAG-1 has also been suggested as a therapeutic target or prognostic marker in breast cancer due to the close association between BAG-1 and functional ER expression.[5] Patients with non-small cell lung cancer (NSCLC) whose tumor exhibited intense BAG-1 cytoplasmic staining appeared to have a better prognosis and this effect was independent of age, stage and histology.[6]

Invitrogen's Mouse anti-BAG-1, Clone 10B6H7 antibody is reactive with BAG-1 as well as the BAG-1M and BAG-1L isoforms.

REAGENT PROVIDED

Mouse anti-BAG-1 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 (NaN_3) as a preservative.

Immunogen: Recombinant human GST-BAG-1 Total protein concentration: g/L

Clone: 10B6H7 Isotype: IgG_1-kappa Antibody concentration: mg/L

STORAGE: 2-8°C

POSITIVE CONTROL TISSUE: Breast carcinoma

EXPECTED STAINING PATTERN: Nucleus and cytoplasm

INSTRUCTIONS FOR USE

PRETREATMENT REQUIREMENTS:

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

Mouse anti-BAG-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.

Invitrogen Corporation • 542 Flynn Rd • Camarillo • CA 93012 • Tel: 800.955.6288 • E-mail: techsupport@invitrogen.com
PIN: 32225 (Rev 08/08) DCC-08-1089

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. Takayama S, et al. *Cancer Res* 58(14):3116-3131, 1998.
2. Tang SC. *IUBMB Life* 53(2):99-105, 2002.
3. Cutress RI, et al. *Br J Cancer* 87(8):834-839, 2002.
4. Tang SC, et al. *Br Cancer Res Treat* 84(3):203-213, 2004.
5. Brimmell M, et al. *Br J Cancer* 81(6):1042-1051, 1999.
6. Rorke S, et al. *Int J Cancer* 95(5):317-322, 2001.

www.invitrogen.com

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

PIN: 32225 (Rev 08/08) DCC-08-1089