

3050 Spruce Street, St. Louis, MO 63103 USA
Tel: (800) 521-8956 (314) 771-5765 Fax: (800) 325-5052 (314) 771-5757
email: techservice@sial.com sigma-aldrich.com

Product Information

Anti-EB1 antibody produced in rabbit

affinity isolated antibody, buffered aqueous solution

Product Number E3406

Product Description

Anti-EB1 is produced in rabbit using a synthetic peptide corresponding to amino acids 252-268 at the C-terminus of human EB1, conjugated to KLH, as immunogen. This sequence is identical in mouse, rat, dog, bovine, and porcine EB1, highly conserved (single amino acid substitution) in chicken EB1, and has limited homology to human EB2 (35% identity) and human EB3 (53% identity). The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-EB1 recognizes EB1. Applications include immunoblotting (35 kDa), immunoprecipitation, and immunofluorescence. Staining of the EB1 band in immunoblotting is specifically inhibited with the immunizing peptide.

Microtubules (MT) play a central role in many cellular functions, including intracellular transportation, maintenance of cell shape and polarity, and chromosome segregation. In most cells, the minus-ends of microtubules are anchored at the centrosome. whereas plus-ends are more dynamic and are responsible for contacting targets such as cell cortex and chromosomes. 1-3 Several factors regulate MT dynamics in cells. EB1 (End-Binding-1) proteins belong to a distinct family of accessory proteins, termed "plus-end tracking proteins" or +TIPs, that bind to the plus-ends of microtubules and are thought to play an important role in regulating dynamics and organization of microtubules. These microtubule-associated proteins (MAPs) are among the most conserved components of the MT cytoskeleton. All EB1 family proteins have a highly conserved N-terminal region that contains a calponin homology (CH) domain and an EB1-like Cterminal motif, which is unique to EB1 family proteins.4 EB1 protein homologs have been identified in many species, conserved from yeast to humans. In humans, there are three genes encoding EB1 family proteins, EB1, EB2 (RP1), and EB3 (EBF3). Human EB1 (35 kDa) family of proteins interact with cytoplasmic microtubules in interphase cells, with mitotic spindles and kinetochores. 5-8 EB1 associates with components of the dynactin complex and cytoplasmic dynein intermediate chain, and interacts with the adenomatous polyposis coli (APC) tumor suppressor gene. 10, 11 EB1 has been shown to bind to the C-

terminus of APC, which implicates EB1 in APC suppression of colonic cancer, and to associate with the mitotic spindle and facilitate spindle assembly throughout the cell cycle. EB2 is localized in the plus ends of microtubule networks in the presence or absence of APC. EB3 associates with the microtubule cytoskeleton and it is predominantly expressed in the central nervous system and preferentially associates with APCL. ¹²

Reagent

Supplied as a solution in 0.01 M phosphate buffered saline, pH 7.4, containing 15 mM sodium azide.

Antibody concentration: ~1.5 mg/mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

For continuous use, store at 2–8 °C for up to one month. For extended storage, freeze in working aliquots. Repeated freezing and thawing, or storage in "frost-free" freezers, is not recommended. If slight turbidity occurs upon prolonged storage, clarify the solution by centrifugation before use. Working dilutions should be discarded if not used within 12 hours.

Product Profile

Immunoblotting: a working concentration of $0.5-1~\mu g/mL$ is recommended using a cell lysate of the rat fibroblast cell line Rat1 or Rat2.

Indirect immunofluorescence: a working concentration of 1–2 $\mu g/mL$ is recommended using Rat1 cells.

Immunoprecipitation: $10-20 \mu g$ of the antibody can immunoprecipitate EB1 from a cell lysate of HeLa cells.

Note: In order to obtain the best results using various techniques and preparations, we recommend determining the optimal working dilutions by titration.

References

- Schuyler, S.C., and Pellman, D., Cell, 105, 421-424 (2001).
- 2. Ou, Y., and Rattner, J.B., *Int. Rev. Cytol.*, **238**, 119-182 (2004).
- 3. Varmark, H., J. Cell Biochem., 91, 904-914 (2004).
- Bu, W., and Su, L-K., J. Biol. Chem., 278, 49721-49731 (2003).
- Berrueta, L., et al., *Proc. Natl. Acad. Sci. USA*, 95, 10596-10601 (1998).
- 6. Piehl, M., and Cassimeris, L., *Mol. Biol. Cell*, **14**, 916-925 (2003).
- Tirnauer, J.S., et al., Mol. Biol. Cell, 13, 4308-4316 (2002).

- 8. Tirnauer, J.S., et al., *J. Cell Biol.*, **149**, 761-766 (2000).
- 9. Berrueta, L., et al., Curr. Biol., 9, 425-428 (1999).
- 10. Su, L. -K., et al., *Cancer Res.*, **55**, 2972-2977 (1995).
- 11. Morrison E.E., et al., *Oncogene*, **17**, 3471-3477 (1998).
- 12. Nakagawa, H., et al., *Oncogene*, **19**, 210-216 (2000).

SG,ER,PHC 08/17-1