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# **ProductInformation**

## Anti-AtEB1b (C-terminal)

produced in rabbit, affinity isolated antibody

Catalog Number E3156

## **Product Description**

Anti-AtEB1b (C-terminal) is developed in rabbit using a synthetic peptide corresponding to amino acids 205-221 located in a region near the C-terminus of *Arabidopsis* AtEB1b, conjugated to KLH, as immunogen. This sequence is highly conserved (single amino acid substitution) in AtEB1a and AtEB1c. The antibody is affinity-purified using the immunizing peptide immobilized on agarose.

Anti-AtEB1 (C-terminal) recognizes AtEB1b fusion protein expressed in transgenic Arabidopsis plants<sup>13</sup> by immunoblotting, 60 kDa. An additional band at 50 kDa may be observed representing AtEB1b degradation products. Staining of AtEB1b in immunoblotting is specifically inhibited with the immunizing peptide.

Microtubules (MT) play a central role in many cellular functions including: intracellular transport, 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 responsible for contacting targets such as cell cortex and chromosomes. 1-3 Several factors regulate MT dynamics in cells. EB1 (End-Binding protein 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 C-terminal motif, which is unique to EB1 family proteins.4 EB1 protein homologs have been identified in many species. In yeast S. cerevisiae, there is only one EB1 gene (BIM1). In Arabidopsis, the EB1 family consists of three members: EB1a, EB1b, and EB1c (30-36 kDa) that show between 82% (EB1a vs. EB1b) and 47% (EB1a/b vs. EB1c) overall amino acid

sequence similarity. <sup>5-8</sup> The N-termini (1-133 amino acids) of EB1a and EB1b are ~93% and ~73% similar, respectively, to the N-terminus of EB1c. In comparison, the C-termini of EB1a and EB1b are ~71% similar and ~32% similar to the C-terminus of EB1c. EB1a and EB1b are associated with growing (+)-ends of acentrosomal plant cortical MT array, whereas EB1c shows nuclear localization during interphase and spindle and phragmoplast localization during mitosis. <sup>9,10</sup> In humans, there are three genes encoding the EB1 family of proteins, EB1, RP1 (EB2), and EBF3 (EB3). Human EB1 family proteins interact with cytoplasmic microtubules in interphase cells, with mitotic spindles and with the adenomatous polyposis coli (APC) tumor suppressor gene. <sup>11, 12</sup>

### Reagent

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

Antibody concentration: ~2 mg/mL

#### **Precautions and Disclaimer**

Due to the sodium azide content, a material safety data sheet (MSDS) for this product has been sent to the attention of the safety officer of your institution. Consult the MSDS for information regarding hazardous 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 1-2  $\mu$ g/mL is recommended using an extract (cytosolic fraction) from the leaves of transgenic Arabidopsis seedlings expressing YFP-taged AtEB1b. <sup>13</sup>

**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).
- Bisgrove, S.R., et al., Plant Physiol., 136, 3855-3863 (2004).
- 6. Akhmanova, A., and Hoogenraad, C.C., *Curr. Opin. Cell Biol.*, **17**, 47-54 (2005).
- 7. Chan, J., et al., Nat. Cell Biol., 5, 967-971 (2003).
- 8. Mathur., J., et al., *Curr. Biol.*, **13**, 1991-1997 (2003).

- 9. Van Damme, D., et al., *Plant J.*, **40**, 386-398 (2004).
- 10. Chan, J., et al., Plant Cell, 17, 1737-1748 (2005).
- 11. Berrueta, L., et al., *Proc. Natl. Acad. Sci. USA*, **95**, 10596-10601 (1998).
- 12. Su, L-K., et al., *Cancer Res.*, **55**, 2972-2977 (1995).
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