



# EB1 siRNA (h): sc-35258

## BACKGROUND

EB1 (end-binding protein 1), also known as microtubule-associated protein RP/EB family member 1 (MAPRE1) or APC-binding protein EB1, may influence tumorigenesis of colorectal cancers and proliferative control of normal cells. EB1 belongs to the intermediate/early gene family, involved in the signal transduction cascade downstream of the T cell receptor (TRC). Colorectal cancer is caused by the pathologic transformation of normal colonic epithelium to an adenomatous polyp, which can become an invasive cancer. APC (adenomatous polyposis coli) is a tumor suppressor gene, the mutation of which is one of the earliest events in colorectal carcinogenesis. A majority of the mutations result in the loss of the carboxy terminus of APC. EB1 has been shown to bind to the carboxy terminal region of APC, which implicates EB1 in APC suppression of colonic cancer. EB1 overexpression may play a role in the development of human esophageal squamous cell carcinoma by affecting APC function and activating the  $\beta$ -catenin/TCF pathway.

## REFERENCES

1. Cottrell, S., et al. 1992. Molecular analysis of APC mutations in familial adenomatous polyposis and sporadic colon carcinomas. *Lancet* 340: 626-630.
2. Su, L.K., et al. 1995. APC binds to the novel protein EB1. *Cancer Res.* 55: 2972-2977.
3. Tirnauer, J.S. and Bierer, B.E. 2000. EB1 proteins regulate microtubule dynamics, cell polarity, and chromosome stability. *J. Cell Biol.* 149: 761-766.
4. Slep, K.C., et al. 2005. Structural determinants for EB1-mediated recruitment of APC and spectraplakins to the microtubule plus end. *J. Cell Biol.* 168: 587-598.

## CHROMOSOMAL LOCATION

Genetic locus: MAPRE1 (human) mapping to 20q11.21.

## PRODUCT

EB1 siRNA (h) is a pool of 3 target-specific 19-25 nt siRNAs designed to knock down gene expression. Each vial contains 3.3 nmol of lyophilized siRNA, sufficient for a 10  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see EB1 shRNA Plasmid (h): sc-35258-SH and EB1 shRNA (h) Lentiviral Particles: sc-35258-V as alternate gene silencing products.

For independent verification of EB1 (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-35258A, sc-35258B and sc-35258C.

## STORAGE AND RESUSPENSION

Store lyophilized siRNA duplex at -20° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at -20° C, avoid contact with RNases and repeated freeze thaw cycles.

Resuspend lyophilized siRNA duplex in 330  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

EB1 siRNA (h) is recommended for the inhibition of EB1 expression in human cells.

## SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29528 (0.3 ml), siRNA Transfection Medium: sc-36868 (20 ml) and siRNA Dilution Buffer: sc-29527 (1.5 ml) are recommended. Control siRNAs or Fluorescein Conjugated Control siRNAs are available as 10  $\mu$ M in 66  $\mu$ l. Each contain a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA. Fluorescein Conjugated Control siRNAs include: sc-36869, sc-44239, sc-44240 and sc-44241. Control siRNAs include: sc-37007, sc-44230, sc-44231, sc-44232, sc-44233, sc-44234, sc-44235, sc-44236, sc-44237 and sc-44238.

## GENE EXPRESSION MONITORING

EB1 (1A11/4): sc-47704 is recommended as a control antibody for monitoring of EB1 gene expression knockdown by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000) or immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500).

To ensure optimal results, the following support reagents are recommended: 1) Western Blotting: use m-IgG $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker™ Molecular Weight Standards: sc-2035, UltraCruz® Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz® Mounting Medium: sc-24941 or UltraCruz® Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor EB1 gene expression knockdown using RT-PCR Primer: EB1 (h)-PR: sc-35258-PR (20  $\mu$ l, 431 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Murawala, P., et al. 2009. Nup358 interacts with APC and plays a role in cell polarization. *J. Cell Sci.* 122: 3113-3322.
2. Chen, Y.T., et al. 2013. Microtubule-associated histone deacetylase 6 supports the calcium store sensor STIM1 in mediating malignant cell behaviors. *Cancer Res.* 73: 4500-4509.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.