SANTA CRUZ BIOTECHNOLOGY, INC.

Raptor siRNA (m): sc-108002

BACKGROUND

Regulatory associated protein of FRAP, also designated Raptor, is a binding partner for mammalian target of Rapamycin kinase (FRAP) and is essential for FRAP signaling *in vivo*. Raptor binding to FRAP is critical for FRAP-catalyzed substrate phosphorylation of 4E-BP1. The Raptor-FRAP complex is nutrient-sensitive and is important for a mechanism by which cells coordinate cell growth and size with changing environmental conditions. Raptor serves as a negative regulator of FRAP kinase activity under nutrient-deprived conditions and is an important component in the FRAP pathway. Raptor is highly expressed in skeletal muscle and to a lesser extent in brain, kidney, lung and placenta.

CHROMOSOMAL LOCATION

Genetic locus: Rptor (mouse) mapping to 11 E2.

PRODUCT

Raptor siRNA (m) 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 µM solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Raptor shRNA Plasmid (m): sc-108002-SH and Raptor shRNA (m) Lentiviral Particles: sc-108002-V as alternate gene silencing products.

For independent verification of Raptor (m) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-108002A, sc-108002B and sc-108002C.

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 µl of the RNAse-free water provided. Resuspension of the siRNA duplex in 330 µl of RNAse-free water makes a 10 µM solution in a 10 µM Tris-HCL, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

Raptor siRNA (m) is recommended for the inhibition of Raptor expression in mouse 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 µM in 66 µl. 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

Raptor (A-2): sc-518004 is recommended as a control antibody for monitoring of Raptor 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-IgGκ BP-HRP: sc-516102 or m-IgGκ 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-IgGκ BP-FITC: sc-516140 or m-IgGκ 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 Raptor gene expression knockdown using RT-PCR Primer: Raptor (m)-PR: sc-108002-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Chin, T.Y., et al. 2010. Inhibition of the mammalian target of Rapamycin promotes cyclic AMP-induced differentiation of NG108-15 cells. Autophagy 6: 1139-1156.
2. Ding, F., et al. 2014. Mammalian target of rapamycin complex 2 signaling pathway regulates transient receptor potential cation channel 6 in podocytes. PLoS ONE 9: e112972.
3. Gao, Y., et al. 2015. Differential IKK/NFκB activity is mediated by TSC2 through mTORC1 in PTEN-null prostate cancer and tuberous sclerosis complex tumor cells. Mol. Cancer Res. 13: 1602-1614.
4. Kusch, A., et al. 2015. 17β-estradiol regulates mTORC2 sensitivity to rapamycin in adaptive cardiac remodeling. PLoS ONE 10: e0123385.
5. Zhang, H.T., et al. 2016. The mTORC2/Akt/NFκB pathway-mediated activation of TRPC6 participates in adriamycin-induced podocyte apoptosis. Cell. Physiol. Biochem. 40: 1079-1093.
6. Pi, H., et al. 2019. Akt inhibition-mediated dephosphorylation of TFE3 promotes overactive autophagy independent of MTORC1 in cadmium-exposed bone mesenchymal stem cells. Autophagy 15: 565-582.
7. Dhar, S.K., et al. 2019. UVB-induced inactivation of manganese-containing superoxide dismutase promotes mitophagy via Ros-mediated mTORC2 pathway activation. J. Biol. Chem. 294: 6831-6842.

RESEARCH USE

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

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.