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TORC2 siRNA (h): sc-45832

BACKGROUND

The TORC (transducer of regulated cAMP response element-binding) proteins, TORC1 and TORC2, are potent CREB coactivators that are exported from the nucleus in a CRM1-dependent manner. The translocation of TORC proteins is a conserved step in the activation of CRE-mediated gene expression induced by cAMP. TORC1 and TORC2 operate via phosphorylation-dependent interactions.

REFERENCES

1. Conkright, M.D., Canettieri, G., Screaton, R., Guzman, E., Miraglia, L., Hogenesch, J.B. and Montminy, M. 2003. TORCs: transducers of regulated CREB activity. *Mol. Cell* 12: 413-423.
2. Iourgenko, V., Zhang, W., Mickanin, C., Daly, I., Jiang, C., Hexham, J.M., Orth, A.P., Miraglia, L., Meltzer, J., Garza, D., Chirn, G.W., McWhinnie, E., Cohen, D., Skelton, J., Terry, R., Yu, Y., Bodian, D., Buxton, F.P., et al. 2003. Identification of a family of cAMP response element-binding protein coactivators by genome-scale functional analysis in mammalian cells. *Proc. Natl. Acad. Sci. USA* 100: 12147-12152.
3. Bittinger, M.A., McWhinnie, E., Meltzer, J., Iourgenko, V., Latario, B., Liu, X., Chen, C.H., Song, C., Garza, D. and Labow, M. 2004. Activation of cAMP response element-mediated gene expression by regulated nuclear transport of TORC proteins. *Curr. Biol.* 14: 2156-2161.
4. Screaton, R.A., Conkright, M.D., Katoh, Y., Best, J.L., Canettieri, G., Jeffries, S., Guzman, E., Niessen, S., Yates, J.R., 3rd, Takemori, H., Okamoto, M. and Montminy, M. 2004. The CREB coactivator TORC2 functions as a calcium- and cAMP-sensitive coincidence detector. *Cell* 119: 61-74.

CHROMOSOMAL LOCATION

Genetic locus: CRT2 (human) mapping to 1q21.3.

PRODUCT

TORC2 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see TORC2 shRNA Plasmid (h): sc-45832-SH and TORC2 shRNA (h) Lentiviral Particles: sc-45832-V as alternate gene silencing products.

For independent verification of TORC2 (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-45832A, sc-45832B and sc-45832C.

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

APPLICATIONS

FcRH2 shRNA Plasmid (h) is recommended for the inhibition of FcRH2 expression in human cells.

SUPPORT REAGENTS

For optimal siRNA transfection efficiency, Santa Cruz Biotechnology's siRNA Transfection Reagent: sc-29527 (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 μ M in 66 μ 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

TORC2 (G-4): sc-166445 is recommended as a control antibody for monitoring of TORC2 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 κ BP-HRP: sc-516102 or m-IgG κ 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 κ BP-FITC: sc-516140 or m-IgG κ 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 TORC2 gene expression knockdown using RT-PCR Primer: TORC2 (h)-PR: sc-45832-PR (20 μ l, 574 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Kim, S.J., Nian, C. and McIntosh, C.H. 2010. GIP increases human adipocyte LPL expression through CREB and TORC2-mediated *trans*-activation of the LPL gene. *J. Lipid Res.* 51: 3145-3157.
2. Samarajeewa, N.U., Docanto, M.M., Simpson, E.R. and Brown, K.A. 2013. CREB-regulated transcription co-activator family stimulates promoter II-driven aromatase expression in preadipocytes. *Horm. Cancer* 4: 233-241.
3. Hill, M.J., Suzuki, S., Segars, J.H. and Kino, T. 2016. CRT2 is a coactivator of GR and couples GR and CREB in the regulation of hepatic gluconeogenesis. *Mol. Endocrinol.* 30: 104-117.

RESEARCH USE

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