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SANTA CRUZ BIOTECHNOLOGY, INC.

GP78 siRNA (m): sc-44579

BACKGROUND

GP78 is the autocrine motility factor (AMF) receptor. AMF (also known as neuroleukin or NLK) is a tumor-secreted cytokine that induces *in vivo* invasion and metastasis. AMF induces tumor cell motility *in vitro* through interaction with GP78. GP78 is distributed evenly across the membranes of normal cells but localizes to the leading and trailing edges of carcinoma cells. In gastric cancer, GP78 surface expression correlates to the pathologic stage and grade of tumor penetration. AMF and GP78 interactions may be involved in a synaptic mechanism for learning and memory formation. GP78 and AMF expression increases in the hippocampi of rats after maze learning. Specifically, GP78 is a RING finger-dependent ubiquitin protein ligase (E3) of the endoplasmic reticulum (ER). GP78 recruits UBC7, an ubiquitin-conjugating enzyme (E2). The E3 activity of GP78 suggests a possible link between metastasis and ubiquitin-mediated protein degradation. In humans, alternative splicing of the GP78 mRNA gives rise to two distinct isoforms, 1 and 2, a type I membrane protein and an integral membrane protein, respectively.

REFERENCES

1. Watanabe, H., et al. 1991. Purification of human tumor cell autocrine motility factor and molecular cloning of its receptor. J. Biol. Chem. 266: 13442-13448.
2. Silletti, S., et al. 1993. Autocrine motility factor receptor in human bladder carcinoma: gene expression, loss of cell-contact regulation and chromosomal mapping. Int. J. Oncol. 3: 801-807.
3. Hirono, Y., et al. 1996. Expression of autocrine motility factor receptor correlates with disease progression in human gastric cancer. Br. J. Cancer 74: 2004-2007.
4. Fang, S., et al. 2001. The tumor autocrine motility factor receptor, GP78, is a ubiquitin protein ligase implicated in degradation from the endoplasmic reticulum. Proc. Natl. Acad. Sci. USA 98: 14422-14427.
5. Luo, Y., et al. 2002. A link between maze learning and hippocampal expression of neuroleukin and its receptor GP78. J. Neurochem. 80: 354-361.

CHROMOSOMAL LOCATION

Genetic locus: Amfr (mouse) mapping to 8 C5.

PRODUCT

GP78 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 GP78 shRNA Plasmid (m): sc-44579-SH and GP78 shRNA (m) Lentiviral Particles: sc-44579-V as alternate gene silencing products.

For independent verification of GP78 (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-44579A, sc-44579B and sc-44579C.

PROTOCOLS

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

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

GP78 siRNA (m) is recommended for the inhibition of GP78-1 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

GP78-1 (F-3): sc-166358 is recommended as a control antibody for monitoring of GP78 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 GP78 gene expression knockdown using RT-PCR Primer: GP78 (m)-PR: sc-44579-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. Kondo, S., et al. 2012. Activation of OASIS family, ER stress transducers, is dependent on its stabilization. Cell Death Differ. 19: 1939-1949.

RESEARCH USE

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