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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

ERK 2 shRNA (m2) Lentiviral Particles: sc-44224-V

BACKGROUND

Mitogen-activated protein kinase (MAPK) signaling pathways involve two closely related MAP kinases, known as extracellular signal-related kinase 1 (ERK 1, p44) and 2 (ERK 2, p42). Growth factors, steroid hormones, G protein-coupled receptor ligands and neurotransmitters can initiate MAPK signaling pathways. Activation of ERK 1 and ERK 2 requires phosphorylation by upstream kinases such as MAP kinase kinase (MEK), MEK kinase and Raf-1. ERK 1 and ERK 2 phosphorylation can occur at specific tyrosine and threonine sites mapping within consensus motifs that include the threonine-glutamate-tyrosine motif. ERK activation leads to dimerization with other ERKs and subsequent localization to the nucleus. Active ERK dimers phosphorylate serine and threonine residues on nuclear proteins and influence a host of responses that include proliferation, differentiation, transcription regulation and development. The human ERK 2 gene maps to chromosome 22q11.21 and encodes a 360 amino acid protein.

REFERENCES

1. Boulton, T.G., et al. 1991. ERKs: a family of protein-serine/threonine kinases that are activated and tyrosine phosphorylated in response to insulin and NGF. *Cell* 65: 663-675.
2. Crews, C.M., et al. 1992. The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. *Science* 258: 478-480.
3. Owaki, H., et al. 1992. Extracellular signal-regulated kinases in T cells: characterization of human ERK 1 and ERK 2 cDNAs. *Biochem. Biophys. Res. Commun.* 182: 1416-1422.
4. Haycock, J.W., et al. 1992. ERK 1 and ERK 2, two microtubule-associated protein 2 kinases, mediate the phosphorylation of tyrosine hydroxylase at Serine 31 *in situ*. *Proc. Natl. Acad. Sci. USA* 89: 2365-2369.
5. Khokhlatchev, A.V., et al. 1998. Phosphorylation of the MAP kinase ERK 2 promotes its homodimerization and nuclear translocation. *Cell* 93: 605-615.
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CHROMOSOMAL LOCATION

Genetic locus: Mapk1 (mouse) mapping to 16 A3.

PRODUCT

ERK 2 shRNA (m2) Lentiviral Particles are concentrated, transduction-ready viral particles containing a target-specific construct that encodes a 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 μ l frozen stock containing 1.0×10^6 infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see ERK 2 siRNA (m2): sc-44224 and ERK 2 shRNA Plasmid (m2): sc-44224-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

ERK 2 shRNA (m2) Lentiviral Particles is recommended for the inhibition of ERK 2 expression in mouse cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 μ l frozen viral stock containing 1.0×10^6 lentiviral transducing particles per milliliter; contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

ERK 2 (D-2): sc-1647 is recommended as a control antibody for monitoring of ERK 2 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ERK 2 gene expression knockdown using RT-PCR Primer: ERK 2 (m2)-PR: sc-44224-PR (20 μ l, 497 bp). Annealing temperature for the primers should be $55-60^\circ$ C and the extension temperature should be $68-72^\circ$ C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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