SANTA CRUZ BIOTECHNOLOGY, INC.

eRF1 siRNA (m): sc-37872

BACKGROUND

Translation is carried out by the ribosome and several associated protein factors through three consecutive steps: initiation, elongation and termination. Termination of protein synthesis takes place when the ribosomal A site is occupied simultaneously by one of three stop codons and by a class 1 translation termination factor. In eukaryotes, this termination factor is the eukaryotic release factor 1 (eRF1), a protein that promotes hydrolysis of the last peptidyl-tRNA on the ribosome. eRF1 activity is stimulated by the association with the GTP-binding protein eRF3. eRF1 forms a quaternary complex with eRF3, GTP and the ribosome. This complex performs a dual role, where, in the "GTP state", it controls the positioning of eRF1 toward the stop codon and peptidyl-tRNA, and, in the "GDP state", it promotes the release of the eRFs from the ribosome. eRF1 contains a highly conserved Asn-Ile-Lys-Ser (NIKS) tetrapeptide, which is essential for the interaction of eRF1 with the ribosome. The gene encoding human eRF1 maps to chromosome 5q31.2.

REFERENCES

1. Frolova, L., et al. 1996. Eukaryotic polypeptide chain release factor eRF3 is an eRF1- and ribosome-dependent guanosine triphosphatase. RNA 2: 334-341.
2. Le Goff, X., et al. 1997. Overexpression of human release factor 1 alone has an antisuppressor effect in human cells. Mol. Cell. Biol. 17: 3164-3172.
3. Frolova, L.Y., et al. 1998. Functional expression of eukaryotic polypeptide chain release factors 1 and 3 by means of baculovirus/insect cells and complex formation between the factors. Eur. J. Biochem. 256: 36-44.
4. Frolova, L., et al. 2002. Highly conserved NIKS tetrapeptide is functionally essential in eukaryotic translation termination factor eRF1. RNA 8: 129-136.
5. Moreira, D., et al. 2002. Evolution of eukaryotic translation elongation and termination factors: variations of evolutionary rate and genetic code deviations. Mol. Biol. Evol. 19: 189-200.
6. Mazur, A.M., et al. 2002. A new method to measure the functional activity of class-1 translation termination factor eRF1. Mol. Biol. 36: 129-135.
7. Dubourg, C., et al. 2002. Evaluation of ETF1/eRF1, mapping to 5q31, as a candidate myeloid tumor suppressor gene. Cancer Genet. Cytogenet. 134: 33-37.

CHROMOSOMAL LOCATION

Genetic locus: Etf1 (mouse) mapping to 18 B1.

PRODUCT

eRF1 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 eRF1 shRNA Plasmid (m): sc-37872-SH and eRF1 shRNA (m) Lentiviral Particles: sc-37872-V as alternate gene silencing products.

For independent verification of eRF1 (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-37872A, sc-37872B and sc-37872C.

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

eRF1 siRNA (m) is recommended for the inhibition of eRF1 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

eRF1 (B-11): sc-365686 is recommended as a control antibody for monitoring of eRF1 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 eRF1 gene expression knockdown using RT-PCR Primer: eRF1 (m)-PR: sc-37872-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.