SANTA CRUZ BIOTECHNOLOGY, INC.

Calmegin shRNA Plasmid (m): sc-60317-SH

BACKGROUND

Calmegin, belonging to the calreticulin family, is expressed in testis as an endoplasmic reticulum membrane protein, where it acts as a chaperone protein and plays a role in spermatogenesis. Calmegin is a single-pass type I membrane protein that is transcriptionally regulated in coordination by CpG methyltransferase and histone deacetylase (HDAC). First expressed in meiotic prophase of spermatocytes, Calmegin facilitates sperm-egg zona pellucida binding through association with sperm membrane proteins (fertilin α and β). A loss in Calmegin results in male sterility. However, if the zona pellucida is partially dissected and fertilized *in vitro,* the egg will develop normally.

REFERENCES

1. Siep, M., Sleddens-Linkels, E., Mulders, S., van Eenennaam, H., Wassenaar, E., Van Cappellen, W.A., Hoogerbrugge, J., Grootegoed, J.A. and Baarends, W.M. 2004. Basic helix-loop-helix transcription factor TCFL5 interacts with the Calmegin gene promoter in mouse spermatogenesis. Nucleic Acids Res. 32: 6425-6436.
2. Nakanishi, T., Isotani, A., Yamaguchi, R., Ikawa, M., Baba, T., Suarez, S.S. and Okabe, M. 2004. Selective passage through the uterotubal junction of sperm from a mixed population produced by chimeras of Calmegin-knockout and wildtype male mice. Biol. Reprod. 71: 959-965.
3. Kim, D.H., Shim, J.S. and Kwon, H.J. 2005. Coordinated transcriptional regulation of Calmegin, a testis-specific molecular chaperon, by histone deacetylase and CpG methyltransferase. Exp. Mol. Med. 37: 492-496.

CHROMOSOMAL LOCATION

Genetic locus: Clgn (mouse) mapping to 8 C2.

PRODUCT

Calmegin shRNA Plasmid (m) is a pool of 3 target-specific lentiviral vector plasmids each encoding 19-25 nt (plus hairpin) shRNAs designed to knock down gene expression. Each plasmid contains a puromycin resistance gene for the selection of cells stably expressing shRNA. Each vial contains 20 µg of lyophilized shRNA plasmid DNA. Suitable for up to 20 transfections. Also see Calmegin siRNA (m): sc-60317 and Calmegin shRNA (m) Lentiviral Particles: sc-60317-V as alternate gene silencing products.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -80° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

PROTOCOLS

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

APPLICATIONS

Calmegin shRNA Plasmid (m) is recommended for the inhibition of Calmegin expression in mouse cells.

SUPPORT REAGENTS

For optimal shRNA Plasmid transfection efficiency, Santa Cruz Biotechnology's shRNA Plasmid Transfection Reagent: sc-108061 (0.2 ml) and shRNA Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control shRNAs are available as 20 µg lyophilized plasmid DNA. Each encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. Control shRNA Plasmids include: sc-108060, sc-108065 and sc-108066.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Calmegin gene expression knockdown using RT-PCR Primer: Calmegin (m)-PR: sc-60317-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

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.