SANTA CRUZ BIOTECHNOLOGY, INC.

CEM15 siRNA (m): sc-60092

BACKGROUND

APOBEC3G is a member of a family of enzymes that have potent DNA mutator activity. APOBEC3G deaminates deoxycytosine to deoxyuracil in the minus strand of HIV-1 DNA, resulting in G to A hypermutation in the plus strand of DNA. Thus, APOBEC3G provides a mechanism for innate immunity to retroviruses and also likely contributes to sequence variation observed in many viruses. Viral infectivity factor (Vif) imparts APOBEC3G resistance to HIV through impaired translation of APOBEC3G mRNA and accelerated posttranslational degradation of APOBEC3G by the 26S proteasome. Interestingly, HIV-1 Vif cannot form a complex with APOBEC3G of mouse origin as it does with the human protein, and thus mouse APOBEC3G functions as a potent inhibitor of wild type HIV-1 replication, where human APOBEC3G is only able to inhibit Vif-deficient HIV-1 replication. This implies that induction of APOBEC3G activity or a method of blocking its interaction with Vif may provide a method for therapeutic intervention. CEM15 is a 429 amino acid mouse protein that is thought to function as an ortholog of human APOBEC3G.

REFERENCES

1. Zhang, H., et al. 2003. The cytidine deaminase CEM15 induces hypermutation in newly synthesized HIV-1 DNA. Nature 424: 94-98.
2. Mangeat, B., et al. 2003. Broad antiretroviral defence by human APOBEC3G through lethal editing of nascent reverse transcripts. Nature 424: 99-103.
3. Shindo, K., et al. 2003. The enzymatic activity of CEM15/Apobec-3G is essential for the regulation of the infectivity of HIV-1 virion but not a sole determinant of its antiviral activity. J. Biol. Chem. 278: 44412-44416.
4. Harris, R.S., et al. 2003. DNA deamination mediates innate immunity to retroviral infection. Cell 113: 803-809.
5. Mariani, R., et al. 2003. Species-specific exclusion of APOBEC3G from HIV-1 virions by Vif. Cell 114: 21-31.
6. Stopak, K., et al. 2003. HIV-1 Vif blocks the antiviral activity of APOBEC3G by impairing both its translation and intracellular stability. Mol. Cell 12: 591-601.
7. Kao, S., et al. 2003. The human immunodeficiency virus type 1 Vif protein reduces intracellular expression and inhibits packaging of APOBEC3G (CEM15), a cellular inhibitor of virus infectivity. J. Virol. 77: 11398-11407.

CHROMOSOMAL LOCATION

Genetic locus: Apobec3 (mouse) mapping to 15 E1.

PRODUCT

CEM15 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 CEM15 shRNA Plasmid (m): sc-60092-SH and CEM15 shRNA (m) Lentiviral Particles: sc-60092-V as alternate gene silencing products.

For independent verification of CEM15 (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-60092A, sc-60092B and sc-60092C.

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

CEM15 siRNA (m) is recommended for the inhibition of CEM15 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

CEM15 (B-2): sc-390254 is recommended as a control antibody for monitoring of CEM15 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 CEM15 gene expression knockdown using RT-PCR Primer: CEM15 (m)-PR: sc-60092-PR (20 µl, 556 bp). 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.