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AID siRNA (m): sc-42730

BACKGROUND

Activation-induced Cytidine Deaminase (AID, HIGM-2) is a 198-amino acid, RNA-editing enzyme that contains a conserved cytidine deaminase motif and plays an important role in B-cell terminal differentiation. AID is expressed in germinal center B cells and contributes to the production of neutralizing antibodies IgG, IgA, and IgE. Hyper-IgM syndrome (HIGM2) patients that have deficient levels of AID show the absence of immuno-globulin class switch recombination (CSR), lack of immuno-globulin somatic hypermutations, and lymph node hyperplasia mediated by the presence of giant germinal centers. Furthermore, AID^{-/-} mice are defective in CSR and also show a hyper-IgM phenotype, characterized by enlarged germinal centers containing active B cells. AID thus appears to be required in several stages of B-cell terminal differentiation that are necessary for efficient antibody responses such as B cell proliferation, immunoglobulin somatic hypermutations and CSR.

REFERENCES

1. Muramatsu, M., et al. 1999. Specific expression of activation-induced cytidine deaminase (AID), a novel member of the RNA-editing deaminase family in germinal center B cells. *J. Biol. Chem.* 274: 18470-18476.
2. Revy, P., et al. 2000. Activation-induced cytidine deaminase (AID) deficiency causes the autosomal recessive form of the Hyper-IgM syndrome (HIGM2). *Cell* 102: 565-575.
3. Muto, T., et al. 2000. Isolation, tissue distribution, and chromosomal localization of the human activation-induced cytidine deaminase (AID) gene. *Genomics* 68: 85-88.
4. Minegishi, Y., et al. 2000. Mutations in activation-induced cytidine deaminase in patients with hyper IgM syndrome. *Clin. Immunol.* 97: 203-210.
5. Muramatsu, M., et al. 2000. Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102: 553-563.

CHROMOSOMAL LOCATION

Genetic locus: *Aicda* (mouse) mapping to 6 F1.

PRODUCT

AID 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see AID shRNA Plasmid (m): sc-42730-SH and AID shRNA (m) Lentiviral Particles: sc-42730-V as alternate gene silencing products.

For independent verification of AID (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-42730A, sc-42730B and sc-42730C.

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 μ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330 μ l of RNase-free water makes a 10 μ M solution in a 10 μ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

APPLICATIONS

AID siRNA (m) is recommended for the inhibition of AID 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 μ M in 66 μ l. 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

AID (ZA001): sc-517548 is recommended as a control antibody for monitoring of AID 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-IgG κ BP-HRP: sc-516102 or m-IgG κ 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-IgG κ BP-FITC: sc-516140 or m-IgG κ 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 AID gene expression knockdown using RT-PCR Primer: AID (m)-PR: sc-42730-PR (20 μ l, 508 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.