SANTA CRUZ BIOTECHNOLOGY, INC.

AP4A Hydrolase siRNA (h): sc-60188

BACKGROUND

Asymmetric diadenosine 5',5'''-P^1 P^4-tetraphosphate (AP4A) hydrolase is a Nudix enzyme that maintains homeostasis by using water to cleave the metabolite AP4A symmetrically back into its original ATP and AMP molecules. AP4A resides in pancreatic β cells where it targets ATP-sensitive K^+ channels and depolarizes the cell membrane causing the excretion of Insulin. AP4A may be involved in the development of diabetes mellitus by raising blood glucose and lowering plasma Insulin. AP4A hydrolase is also active towards other adenosine and diadenosine polyphosphates with four or more phosphate groups, but not towards diadenosine triphosphate. AP4A always generates ATP as one of the products. AP4A hydrolase is involved in heat shock and metabolic stress by regulating intracellular dinucleoside polyphosphate concentrations.

REFERENCES

1. Abdelghany, H.M., et al. 2001. Cloning, characterisation and crystallisation of a diadenosine 5',5'''-P^1,P^4-tetraphosphate pyrophosphohydrolase from *Caenorhabditis elegans*. Biochim. Biophys. Acta 1550: 27-36.
2. Fletcher, J.I., et al. 2002. The structure of AP4A hydrolase complexed with ATP-MgF_x reveals the basis of substrate binding. Structure 10: 205-213.
3. Bailey, S., et al. 2002. The crystal structure of diadenosine tetraphosphate hydrolase from *Caenorhabditis elegans* in free and binary complex forms. Structure 10: 589-600.
4. Stavrou, B.M. 2003. Diadenosine polyphosphates: postulated mechanisms mediating the cardiac effects. Curr. Med. Chem. Cardiovasc. Hematol. Agents 1: 151-169.
5. Rüsing, D. and Verspohl, E.J. 2004. Influence of diadenosine tetraphosphate (AP4A) on lipid metabolism. Cell Biochem. Funct. 22: 333-338.
6. Swarbrick, J.D., et al. 2005. ^{1}H, ^{13}C, and ^{15}N resonance assignments of the 17 kDa AP4A hydrolase from *Homo sapiens* in the presence and absence of ATP. J. Biomol. NMR 31: 181-182.
7. Soto, D., et al. 2005. Effects of dinucleoside polyphosphates on trabecular meshwork cells and aqueous humor outflow facility. J. Pharmacol. Exp. Ther. 314: 1042-1051.

CHROMOSOMAL LOCATION

Genetic locus: NUDT2 (human) mapping to 9p13.3.

PRODUCT

AP4A Hydrolase siRNA (h) 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 AP4A Hydrolase shRNA Plasmid (h): sc-60188-SH and AP4A Hydrolase shRNA (h) Lentiviral Particles: sc-60188-V as alternate gene silencing products.

For independent verification of AP4A Hydrolase (h) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-60188A, sc-60188B and sc-60188C.

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

AP4A Hydrolase siRNA (h) is recommended for the inhibition of AP4A Hydrolase expression in human 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

AP4A Hydrolase (F-5): sc-271410 is recommended as a control antibody for monitoring of AP4A Hydrolase 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 AP4A Hydrolase gene expression knockdown using RT-PCR Primer: AP4A Hydrolase (h)-PR: sc-60188-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.