SANTA CRUZ BIOTECHNOLOGY, INC.

AMCase siRNA (m): sc-60161

BACKGROUND

Chitinases are ubiquitous chitin-fragmenting hydrolases. The chitinase Chitotriosidase is capable of cleaving natural chitin and chitin-like substrates in humans and may play a role in immunity against pathogens containing chitin. Activated human macrophages secrete chitotriosidase, and increased plasma levels of chitotriosidase are a feature of patients suffering from Gaucher's disease. Expression of mouse Chitotriosidase is restricted to brain, skin, bone marrow, kidney, tongue, stomach and testis. The homology between chito-triosidase and chitinases found in lower organisms is significant. Acidic mammalian chitinase precursor (AMCase) degrades chitotriose and chitin. AMCase is highly expressed in stomach tissues and is primarily a secreted protein. It is involved in Th2-mediated inflammation and may play a role in asthma and allergic diseases.

REFERENCES

1. Zhu, Z., et al. 2004. Acidic mammalian chitinase in asthmatic Th2 inflammation and IL-13 pathway activation. Science 304: 1678-1682.
2. Malaguarnera, L., et al. 2005. Interferon-γ, tumor necrosis factor-α, and lipopolysaccharide promote chitotriosidase gene expression in human macrophages. J. Clin. Lab. Anal. 19: 128-132.
3. Di Rosa, M., et al. 2005. Effect of interferon-γ, interleukin-10, lipopolysaccharide and tumor necrosis factor-α on chitotriosidase synthesis in human macrophages. Clin. Chem. Lab. Med. 43: 499-502.
4. Aerts, J.M., et al. 2005. Identification and use of biomarkers in Gaucher disease and other lysosomal storage diseases. Acta Paediatr. Suppl. 94: 43-46.
5. Deegan, P.B., et al. 2005. Clinical evaluation of biomarkers in Gaucher disease. Acta Paediatr. Suppl. 94: 47-50.
6. Brinkman, J., et al. 2005. Plasma chitotriosidase and CCL18: early biochemical surrogate markers in type B Niemann-Pick disease. J. Inherit. Metab. Dis. 28: 13-20.
7. Boot, R.G., et al. 2005. Marked differences in tissue-specific expression of chitinases in mouse and man. J. Histochem. Cytochem. 53: 1283-1292.
8. Chou, Y.T., et al. 2006. Kinetic characterization of recombinant human acidic mammalian chitinase. Biochemistry 45: 4444-4454.

CHROMOSOMAL LOCATION

Genetic locus: Chia (mouse) mapping to 3 F2.2.

PRODUCT

AMCase 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 AMCase shRNA Plasmid (m): sc-60161-SH and AMCase shRNA (m) Lentiviral Particles: sc-60161-V as alternate gene silencing products.

For independent verification of AMCase (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-60161A, sc-60161B and sc-60161C.

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

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

AMCase (E-11): sc-365676 is recommended as a control antibody for monitoring of AMCase 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 AMCase gene expression knockdown using RT-PCR Primer: AMCase (m)-PR: sc-60161-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.