

Muted siRNA (h): sc-61108

BACKGROUND

Biogenesis of lysosome-related organelles complex-1 (BLOC-1) is a multisubunit protein necessary for biogenesis of specialized organelles of the endosomal-lysosomal system (such as melanosomes and platelet-dense granules). The complex consists of coiled-coil-forming proteins Snapin, Pallidin, Cappuccino, Muted, BLOS1, BLOS2, and BLOS3. The localization of these proteins varies as they can be cytoplasmic, peripheral membrane bound or anchored to the vesicular membrane. The Muted (mu) mouse is a model for Hermansky-Pudlak syndrome (HPS), an inherited disorder in humans causing hypopigmentation, hemorrhaging and early death due to lung abnormalities. The Muted gene is ubiquitously expressed with higher levels in brain, bone marrow, kidney, and liver and lower levels in skeletal muscle.

REFERENCES

1. Zhang, Q., et al. 2002. The gene for the muted (mu) mouse, a model for Hermansky-Pudlak syndrome, defines a novel protein which regulates vesicle trafficking. *Hum. Mol. Genet.* 11: 697-706.
2. Li, W., et al. 2003. Hermansky-Pudlak syndrome type 7 (HPS-7) results from mutant dysbindin, a member of the biogenesis of lysosome-related organelles complex 1 (BLOC-1). *Nat. Genet.* 35: 84-89.
3. Ciciotte, S.L., et al. 2003. Cappuccino, a mouse model of Hermansky-Pudlak syndrome, encodes a novel protein that is part of the pallidin-muted complex (BLOC-1). *Blood* 101: 4402-4407.
4. Gwynn, B., et al. 2004. Reduced pigmentation (rp), a mouse model of Hermansky-Pudlak syndrome, encodes a novel component of the BLOC-1 complex. *Blood* 104: 3181-3189.
5. Starcevic, M., et al. 2004. Identification of snapin and three novel proteins (BLOS1, BLOS2, and BLOS3/reduced pigmentation) as subunits of biogenesis of lysosome-related organelles complex-1 (BLOC-1). *J. Biol. Chem.* 279: 28393-28401.
6. Bossi, G., et al. 2005. Normal lytic granule secretion by cytotoxic T lymphocytes deficient in BLOC-1, -2 and -3 and myosins Va, VIIa and XV. *Traffic* 6: 243-251.

CHROMOSOMAL LOCATION

Genetic locus: BLOC1S5 (human) mapping to 6p24.3.

PRODUCT

Muted 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 μ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see Muted shRNA Plasmid (h): sc-61108-SH and Muted shRNA (h) Lentiviral Particles: sc-61108-V as alternate gene silencing products.

For independent verification of Muted (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-61108A, sc-61108B and sc-61108C.

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

Muted siRNA (h) is recommended for the inhibition of Muted 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 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

Muted (F-4): sc-393554 is recommended as a control antibody for monitoring of Muted 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 Muted gene expression knockdown using RT-PCR Primer: Muted (h)-PR: sc-61108-PR (20 μ l). 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.