

GW182 siRNA (h): sc-45516

BACKGROUND

GW bodies (GWBs) function as storage centers and degradation sites for mRNAs. GWBs are crucial intracellular structures for miRNA function. Disassembly or disruption of GWBs has been shown to impair siRNA and miRNA silencing activity. GW182 is a cytoplasmic marker protein for GWBs. GW182 autoantigen, also designated EMSY interactor protein, plays a role in the maintenance and stability of the GWB structures. GW182 is an ubiquitously expressed protein that binds to mRNA. The GW182 protein may interact with endogenous argonaute-2 (Ago2), which is also enriched in GWBs. The GW182 protein is detected in patients with Ataxia, Sjogren's syndrome (SS) and sensor neuropathy disease, who develop autoantibodies against GWB structure proteins.

REFERENCES

1. Eystathiou, T., et al. 2002. A phosphorylated cytoplasmic autoantigen, GW182, associates with a unique population of human mRNAs within novel cytoplasmic speckles. *Mol. Biol. Cell* 13: 1338-1351.
2. Eystathiou, T., et al. 2003. Clinical and serological associations of autoantibodies to GW bodies and a novel cytoplasmic autoantigen GW182. *J. Mol. Med.* 81: 811-818.
3. Eystathiou, T., et al. 2003. The GW182 protein colocalizes with mRNA degradation associated proteins hDcp1 and hLSm4 in cytoplasmic GW bodies. *RNA* 9: 1171-1173.
4. Eystathiou, T., et al. 2003. A panel of monoclonal antibodies to cytoplasmic GW bodies and the mRNA binding protein GW182. *Hybrid Hybridomics* 22: 79-86.

CHROMOSOMAL LOCATION

Genetic locus: TNRC6A (human) mapping to 16p12.1.

PRODUCT

GW182 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 GW182 shRNA Plasmid (h): sc-45516-SH and GW182 shRNA (h) Lentiviral Particles: sc-45516-V as alternate gene silencing products.

For independent verification of GW182 (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-45516A, sc-45516B and sc-45516C.

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

GW182 siRNA (h) is recommended for the inhibition of GW182 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

GW182 (A-6): sc-374458 is recommended as a control antibody for monitoring of GW182 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 GW182 gene expression knockdown using RT-PCR Primer: GW182 (h)-PR: sc-45516-PR (20 μ l, 598 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

1. Bukong, T.N., et al. 2013. Ethanol facilitates hepatitis C virus replication via up-regulation of GW182 and heat shock protein 90 in human hepatoma cells. *Hepatology* 57: 70-80.

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.