SANTA CRUZ BIOTECHNOLOGY, INC.

cyt19 siRNA (h): sc-60494

BACKGROUND

Formation of methylated metabolites is a critical step in the metabolism of inorganic arsenic. Arsenite methyltransferase (cyt19) is localized to the cytoplasm and operates in the transfer of a methyl group from AdoMet to trivalent arsenicals producing methylated and dimethylated arsenicals. It methylates arsenite to form methylarsonate which is reduced to methylarsonite. Methylarsonite acts as a substrate and is converted into a much less toxic compound dimethylarsinate. Cyt19 is highly expressed in liver. Inherited variation in cyt19 may contribute to variation in arsenic metabolism and possibly arsenic-dependent carcinogenesis in humans.

REFERENCES

1. Walton, F.S., et al. 2003. Selenium compounds modulate the activity of recombinant rat AsIII-methyl and the methylation of arsenite by rat and human hepatocytes. Chem. Res. Toxicol. 16: 261-265.
2. Waters, S.B., et al. 2004. Endogenous reductants support the catalytic function of recombinant rat cyt19, an arsenic methyltransferase. Chem. Res. Toxicol. 17: 404-409.
3. Thomas, D.J., et al. 2004. Elucidating the pathway for arsenic methylation. Toxicol. Appl. Pharmacol. 198: 319-326.
4. Drobn, Z., et al. 2004. Interindividual variation in the metabolism of arsenic in cultured primary human hepatocytes. Toxicol. Appl. Pharmacol. 201: 166-177.
5. Hayakawa, T., et al. 2005. A new metabolic pathway of arsenite: arsenic-glutathione complexes are substrates for human arsenic methyltransferase cyt19. Arch. Toxicol. 79: 183-191.
6. Meza, M.M., et al. 2005. Developmentally restricted genetic determinants of human arsenic metabolism: association between urinary methylated arsenic and cyt19 polymorphisms in children. Environ. Health Perspect. 113: 775-781.
7. Wood, T.C., et al. 2006. Human arsen and functional genomics studies. J. Biol. Chem. 281: 7364-7373.

CHROMOSOMAL LOCATION

Genetic locus: AS3MT (human) mapping to 10q24.32.

PRODUCT

cyt19 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 cyt19 shRNA Plasmid (h): sc-60494-SH and cyt19 shRNA (h) Lentiviral Particles: sc-60494-V as alternate gene silencing products.

For independent verification of cyt19 (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-60494A, sc-60494B and sc-60494C.

RESEARCH USE

For research use only, not for use in diagnostic procedures.

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

cyt19 siRNA (h) is recommended for the inhibition of cyt19 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

cyt19 (F-9): sc-377436 is recommended as a control antibody for monitoring of cyt19 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 cyt19 gene expression knockdown using RT-PCR Primer: cyt19 (h)-PR: sc-60494-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.