SANTA CRUZ BIOTECHNOLOGY, INC.

TPMT siRNA (h): sc-61701

BACKGROUND

Thiopurine S-methyltransferase (TPMT), also designated thiopurine methyltransferase, acts as a catalyst for the S-methylation of thiopurine drugs such as 6-mercaptopurine. TPMT, usually found as a monomer, is inhibited by S-adenosyl-L-homocysteine. It is a cytoplasmic protein belongs to the TPMT subfamily of the larger methyltransferase superfamily of proteins. TPMT activity varies among different ethnic groups, with a large majority of Caucasians having a high TPMT activity. A common genetic polymorphism controls the level of TPMT activity. The level of TPMT activity is associated with the variation in efficacy and toxicity of thiopurine drugs.

REFERENCES

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2. Ford, L., et al. 2004. Whose TPMT activity is it anyway? Ann. Clin. Biochem. 41: 498-500.
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4. Okada, Y., et al. 2005. Genotyping of thiopurine methyltransferase using pyrosequencing. Biol. Pharm. Bull. 28: 677-681.
5. Heckmann, J.M., et al. 2005. Thiopurine methyltransferase (TPMT) heterozygosity and enzyme activity as predictive tests for the development of azathioprine-related adverse events. J. Neurol. Sci. 231: 71-80.
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7. Sayani, F.A., et al. 2005. Thiopurine methyltransferase enzyme activity determination before treatment of inflammatory bowel disease with azathioprine: effect on cost and adverse events. Can. J. Gastroenterol. 19: 147-151.
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CHROMOSOMAL LOCATION

Genetic locus: TPMT (human) mapping to 6p22.3.

PRODUCT

TPMT 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 TPMT shRNA Plasmid (h): sc-61701-SH and TPMT shRNA (h) Lentiviral Particles: sc-61701-V as alternate gene silencing products.

For independent verification of TPMT (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-61701A, sc-61701B and sc-61701C.

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

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

TPMT (E-8): sc-374154 is recommended as a control antibody for monitoring of TPMT 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 TPMT gene expression knockdown using RT-PCR Primer: TPMT (h)-PR: sc-61701-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.