SANTA CRUZ BIOTECHNOLOGY, INC.

pHyde siRNA (h): sc-37487

BACKGROUND

Caspases modulate apoptosis in various ways. Specifically, caspase-3, a death protease, is instrumental in cleaving cellular proteins, dismantling the cell and forming apoptotic bodies. pHyde has a potential role as a tumor suppressor by inducing caspase 3-mediated apoptosis and stimulating p53 expression. A dose-dependent increase in caspase-3 activity is observed in transduced pHyde DU145 cells. Furthermore, caspase-3 may be necessary for pHyde-mediated apoptosis. The pHyde gene may up-regulate the apoptosis pathway and thus have a potential application in cancer gene therapy. Recombinant pHyde inhibits the growth of human prostate cancer cell lines DU145 and LNCaP *in vitro*. DU145 tumors may be reduced significantly *in vivo* when nude mice are injected with recombinant pHyde. pHyde also has a demonstrated growth inhibitory effect on human breast cancer cells. This suggests that pHyde may have a role in inhibiting different tumor types.

REFERENCES

1. Porter, A.G., et al. 1999. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 6: 99-104.
2. Slee, E.A., et al. 1999. Serial killers: ordering caspase activation events in apoptosis. Cell Death Differ. 6: 1067-1074.
3. Steiner, M.S., et al. 2000. Growth inhibition of prostate cancer by an adenovirus expressing a novel tumor suppressor gene, pHyde. Cancer Res. 60: 4419-4425.
4. Rinaldy, A.R., et al. 2000. Role of pHyde novel gene product as an intrinsic factor for apoptotic pathway in prostate cancer. Gan To Kagaku Ryoho 2: 215-222.
5. Zhang, X., et al. 2001. Apoptosis induction in prostate cancer cells by a novel gene product, pHyde, involves caspase-3. Oncogene 20: 5982-5990.
6. Ohgami, R.S., et al. 2006. The Steap proteins are metalloreductases. Blood 108: 1388-1394.
7. Knutson, M.D. 2007. Steap proteins: implications for iron and copper metabolism. Nutr. Rev. 65: 335-340.

CHROMOSOMAL LOCATION

Genetic locus: STEAP3 (human) mapping to 2q14.2.

PRODUCT

pHyde 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 pHyde shRNA Plasmid (h): sc-37487-SH and pHyde shRNA (h) Lentiviral Particles: sc-37487-V as alternate gene silencing products.

For independent verification of pHyde (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-37487A, sc-37487B and sc-37487C.

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

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

pHyde (H-4): sc-376327 is recommended as a control antibody for monitoring of pHyde 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 pHyde gene expression knockdown using RT-PCR Primer: pHyde (h)-PR: sc-37487-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.