

AT₁ siRNA (r): sc-155992

BACKGROUND

Angiotensin II (Ang II) is an important physiological effector of blood pressure and volume regulation through vasoconstriction, aldosterone release, sodium uptake and thirst stimulation. Although Ang II interacts with two types of cell surface receptors, AT₁ and AT₂, most of the major cardiovascular effects seem to be mediated through AT₁. Molecular cloning of the AT₁ protein has shown it to be a member of the G protein-associated seven transmembrane protein receptor family. Ang II treatment of cells results in activation of several signal transduction pathways as evidenced by tyrosine phosphorylation of several proteins and induction of others. PLC γ is phosphorylated after 30 seconds of treatment with Angiotensin II, indicating this as an early signal transduction event. Ang II treatment also stimulates phosphorylation of Shc, FAK and MAP kinases, and induces MKP-1, indicating stimulation of growth factor pathways. Ang II stimulation through AT₁ has been shown to activate the JAK/Stat pathway involving a direct interaction between JAK2 and AT₁ as demonstrated by coimmunoprecipitation. The AT₁ receptor has no cytoplasmic kinase domain, but is able to function as a substrate for Src kinases and has several putative phosphorylation sites.

REFERENCES

- Murphy, T.J., et al. 1991. Isolation of a cDNA encoding the vascular type-1 Angiotensin II receptor. *Nature* 351: 233-236.
- Tsuda, T., et al. 1991. Vasoconstrictor-induced protein-tyrosine phosphorylation in cultured vascular smooth muscle cells. *FEBS Lett.* 285: 44-48.
- Duff, J.L., et al. 1993. Angiotensin II induces 3CH134, a protein-tyrosine phosphatase, in vascular smooth muscle cells. *J. Biol. Chem.* 268: 26037-26040.

CHROMOSOMAL LOCATION

Genetic locus: Agtr1b (rat) mapping to 2q24.

PRODUCT

AT₁ siRNA (r) 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 AT₁ shRNA Plasmid (r): sc-155992-SH and AT₁ shRNA (r) Lentiviral Particles: sc-155992-V as alternate gene silencing products.

For independent verification of AT₁ (r) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-155992A, sc-155992B and sc-155992C.

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

AT₁ siRNA (r) is recommended for the inhibition of AT₁ expression in rat 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

AT₁ (G-3): sc-515884 is recommended as a control antibody for monitoring of AT₁ 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 AT₁ gene expression knockdown using RT-PCR Primer: AT₁ (r)-PR: sc-155992-PR (20 μ l, 592 bp). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

SELECT PRODUCT CITATIONS

- Sandoval, Y.H., et al. 2011. Transactivation of epidermal growth factor receptor by enhanced levels of endogenous angiotensin II contributes to the overexpression of G_i α proteins in vascular smooth muscle cells from SHR. *Cell. Signal.* 23: 1716-1726.
- Lyu, J., et al. 2018. Angiotensin II induces cholesterol accumulation and impairs Insulin secretion by regulating ABCA1 in β cells. *J. Lipid Res.* 59: 1906-1915.

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.