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AT₂ siRNA (r): sc-156014

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.
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- 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.
- Timmermans, P.B., et al. 1993. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol. Rev.* 45: 205-251.
- Marrero, M.B., et al. 1994. Angiotensin II stimulates tyrosine phosphorylation of phospholipase C- γ 1 in vascular smooth muscle cells. *J. Biol. Chem.* 269: 10935-10939.
- Schorb, W., et al. 1994. Angiotensin II-induced protein tyrosine phosphorylation in neonatal rat cardiac fibroblasts. *J. Biol. Chem.* 269: 19626-19632.

CHROMOSOMAL LOCATION

Genetic locus: Agtr2 (rat) mapping to Xq34.

PRODUCT

AT₂ 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 AT₂ shRNA Plasmid (r): sc-156014-SH and AT₂ shRNA (r) Lentiviral Particles: sc-156014-V as alternate gene silencing products.

For independent verification of AT₂ (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-156014A, sc-156014B and sc-156014C.

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

AT₂ siRNA (r) is recommended for the inhibition of AT₂ 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.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor AT₂ gene expression knockdown using RT-PCR Primer: AT₂ (r)-PR: sc-156014-PR (20 μ l). 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.