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SZABO-SCANDIC HandelsgmbH

Quellenstraße 110, A-1100 Wien

T. +43(0)1 489 3961-0

F. +43(0)1 489 3961-7

mail@szabo-scandic.com

www.szabo-scandic.com

[linkedin.com/company/szaboscandic](https://www.linkedin.com/company/szaboscandic) 

Annexin V shRNA (h2) Lentiviral Particles: sc-44282-V

BACKGROUND

The Annexin family of calcium-binding proteins is composed of at least ten mammalian genes and is characterized by a conserved core domain, which binds phospholipids in a Ca²⁺-dependent manner, and a unique amino-terminal region, which may confer binding specificity. Annexin family members have been implicated as regulators of such diverse processes as ion flux, endocytosis and exocytosis, and cellular adhesion. For example, the crystal structure of Annexin III has suggested a hydrophilic amino-terminus with possible Ca²⁺ channel activity. Similarly, Annexin V has ion channel properties. Annexin IV, also referred to as endonexin, functions to regulate Cl⁻ flux by mediating calmodulin kinase II (CaMKII) activity and Annexin V has been shown to regulate PKC activity. Annexin V is ubiquitously expressed at high levels in tissues and cells grown in tissue culture, while Annexin VIII exhibits a more limited distribution. Where co-expressed in the same tissues, Annexin VIII is often expressed at a 100-fold lower level than Annexin V. However, Annexin VIII is preferentially expressed in acute promyelocytic leukemia (APL) cells, which may relate to its role in hematopoietic cell differentiation.

REFERENCES

1. Smith, P.D., et al. 1994. Structural evolution of the Annexin supergene family. *Trends Gen.* 10: 241-246.
2. Chan, H.C., et al. 1994. Annexin IV inhibits calmodulin-dependent protein kinase II-activated chloride conductance. A novel mechanism for ion channel regulation. *J. Biol. Chem.* 269: 32464-32468.
3. Reutelingsperger, C.P., et al. 1994. Differential tissue expression of Annexin VIII in human. *FEBS Lett.* 349: 120-124.
4. Liu, J.H., et al. 1994. Expression of the Annexin VIII gene in acute promyelocytic leukemia. *Leuk. Lymphoma* 13: 381-386.
5. Rothhut, B., et al. 1995. Inhibitory effect of Annexin V on protein kinase C activity in mesangial cell lysates. *Eur. J. Biochem.* 232: 865-872.

CHROMOSOMAL LOCATION

Genetic locus: ANXA5 (human) mapping to 4q27.

PRODUCT

Annexin V shRNA (h2) Lentiviral Particles is a pool of concentrated, transduction-ready viral particles containing 3 target-specific constructs that encode 19-25 nt (plus hairpin) shRNA designed to knock down gene expression. Each vial contains 200 µl frozen stock containing 1.0 x 10⁶ infectious units of virus (IFU) in Dulbecco's Modified Eagle's Medium with 25 mM HEPES pH 7.3. Suitable for 10-20 transductions. Also see Annexin V siRNA (h2): sc-44282 and Annexin V shRNA Plasmid (h2): sc-44282-SH as alternate gene silencing products.

STORAGE

Store lentiviral particles at -80° C. Stable for at least one year from the date of shipment. Once thawed, particles can be stored at 4° C for up to one week. Avoid repeated freeze thaw cycles.

APPLICATIONS

Annexin V shRNA (h2) Lentiviral Particles is recommended for the inhibition of Annexin V expression in human cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10⁶ infectious units of virus (IFU); contains an shRNA construct encoding a scrambled sequence that will not lead to the specific degradation of any known cellular mRNA.

GENE EXPRESSION MONITORING

Annexin V (A-2): sc-393669 is recommended as a control antibody for monitoring of 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 (secondary) reagents are recommended: 1) Western Blotting: use goat anti-mouse IgG-HRP: sc-2005 (dilution range: 1:2000-1:32,000) or Cruz Marker™ compatible goat anti-mouse IgG-HRP: sc-2031 (dilution range: 1:2000-1:5000), Cruz Marker™ Molecular Weight Standards: sc-2035, TBS Blotto A Blocking Reagent: sc-2333 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use goat anti-mouse IgG-FITC: sc-2010 (dilution range: 1:100-1:400) or goat anti-mouse IgG-TR: sc-2781 (dilution range: 1:100-1:400) with UltraCruz™ Mounting Medium: sc-24941.

RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor Annexin V gene expression knockdown using RT-PCR Primer: Annexin V (h2)-PR: sc-44282-PR (20 µl). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

BIOSAFETY

Lentiviral particles can be employed in standard Biosafety Level 2 tissue culture facilities (and should be treated with the same level of caution as with any other potentially infectious reagent). Lentiviral particles are replication-incompetent and are designed to self-inactivate after transduction and integration of shRNA constructs into genomic DNA of target cells.

RESEARCH USE

The purchase of this product conveys to the buyer the nontransferable right to use the purchased amount of the product and all replicates and derivatives for research purposes conducted by the buyer in his laboratory only (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party, or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes.

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