

# ATG5 siRNA (bovine): sc-270383

## BACKGROUND

In yeast, autophagy is an essential process for survival during nutrient starvation and cell differentiation. The process of autophagy is characterized as a non-selective degradation of cytoplasmic proteins into membrane structures called autophagosomes, and it is dependent on several proteins, including the autophagy proteins ATG5 (APG5) and APG7. Yeast Apg7 and the human homolog, APG7, share similarities with the ubiquitin-activating enzyme E1 in *Saccharomyces cerevisiae* and are likewise responsible for enzymatically activating the autophagy conjugation system. Apg5 and the human homolog, ATG5, also designated APG5, apoptosis-specific protein or APS, function as substrates for the autophagy protein Apg12. These proteins are covalently bonded together to form Apg12/ATG5 conjugates, which are required for the progression of autophagy.

## REFERENCES

1. Kametaka, S., et al. 1996. Structural and functional analyses of APG5, a gene involved in autophagy in yeast. *Gene* 178: 139-143.
2. Mizushima, N., et al. 1998. A protein conjugation system essential for autophagy. *Nature* 395: 395-398.
3. Hammond, E.M., et al. 1998. Homology between a human apoptosis specific protein and the product of APG5, a gene involved in autophagy in yeast. *FEBS Lett.* 425: 391-395.
4. Klionsky, D.J. 1998. Nonclassical protein sorting to the yeast vacuole. *J. Biol. Chem.* 273: 10807-10810.

## CHROMOSOMAL LOCATION

Genetic locus: ATG5 (bovine) mapping to 9.

## PRODUCT

ATG5 siRNA (bovine) 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  $\mu$ M solution once resuspended using protocol below. Suitable for 50-100 transfections. Also see ATG5 shRNA Plasmid (bovine): sc-270383-SH and ATG5 shRNA (bovine) Lentiviral Particles: sc-270383-V as alternate gene silencing products.

For independent verification of ATG5 (bovine) gene silencing results, we also provide the individual siRNA duplex components. Each is available as 3.3 nmol of lyophilized siRNA. These include: sc-270383A, sc-270383B and sc-270383C.

## 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  $\mu$ l of the RNase-free water provided. Resuspension of the siRNA duplex in 330  $\mu$ l of RNase-free water makes a 10  $\mu$ M solution in a 10  $\mu$ M Tris-HCl, pH 8.0, 20 mM NaCl, 1 mM EDTA buffered solution.

## APPLICATIONS

ATG5 siRNA (bovine) is recommended for the inhibition of ATG5 expression in bovine 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  $\mu$ M in 66  $\mu$ 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

ATG5 (C-1): sc-133158 is recommended as a control antibody for monitoring of ATG5 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 $\kappa$  BP-HRP: sc-516102 or m-IgG $\kappa$  BP-HRP (Cruz Marker): sc-516102-CM (dilution range: 1:1000-1:10000), Cruz Marker<sup>™</sup> Molecular Weight Standards: sc-2035, UltraCruz<sup>®</sup> Blocking Reagent: sc-516214 and Western Blotting Luminol Reagent: sc-2048. 2) Immunofluorescence: use m-IgG $\kappa$  BP-FITC: sc-516140 or m-IgG $\kappa$  BP-PE: sc-516141 (dilution range: 1:50-1:200) with UltraCruz<sup>®</sup> Mounting Medium: sc-24941 or UltraCruz<sup>®</sup> Hard-set Mounting Medium: sc-359850.

## RT-PCR REAGENTS

Semi-quantitative RT-PCR may be performed to monitor ATG5 gene expression knockdown using RT-PCR Primer: ATG5 (bovine)-PR: sc-270383-PR (20  $\mu$ l). Annealing temperature for the primers should be 55-60° C and the extension temperature should be 68-72° C.

## SELECT PRODUCT CITATIONS

1. Du, J., et al. 2012. Role of autophagy in angiogenesis in aortic endothelial cells. *Am. J. Physiol., Cell Physiol.* 302: C383-C391.

## RESEARCH USE

For research use only, not for use in diagnostic procedures.

## PROTOCOLS

See our web site at [www.scbt.com](http://www.scbt.com) for detailed protocols and support products.