SANTA CRUZ BIOTECHNOLOGY, INC.

Control shRNA Plasmid-B: sc-108065

BACKGROUND

RNA interference (RNAi) is one of the most exciting discoveries of the past decade in functional genomics and proteomics. While first recognized in nematodes as a response to exogenously introduced long double-stranded RNA (dsRNA), it is now clear that RNAi is utilized by most eukaryotes *in vivo* for anti-viral defense, transposon activity modulation and gene regulation, and has rapidly become an important research tool for gene silencing. Specifically, RNAi is the pathway by which short interfering RNA (siRNA) or short hairpin RNA (shRNA) are used to silence the expression of target genes. Compared to siRNA, shRNA offers advantages in silencing longevity and delivery.

Upon introduction, the shRNA plasmid DNA enters the cell where shRNA is transcribed. The shRNA is then cleaved by an RNase III-like enzyme called Dicer into small interfering RNA (siRNA), which are short RNA duplexes of 19-21 nucleotides with two nucleotide 3' overhangs on each strand. The siRNAs are then assembled into endoribonuclease-containing complexes known as RNA-induced silencing complexes (RISCs), unwinding in the process. Activated RISCs subsequently bind to complementary transcripts by base pairing interactions between the siRNA anti-sense strand and complementary mRNA. The bound mRNA is cleaved and sequence specific degradation of mRNA results in gene silencing. In mammalian cells, introduction of long dsRNA (more than 30 nucleotides) initiates a potent anti-viral response, exemplified by nonspecific inhibition of protein synthesis and RNA degradation. The mammalian anti-viral response can be bypassed, however, by the introduction of siRNAs or shRNA plasmid DNA.

Santa Cruz Biotechnology, Inc. currently offers more than 49,000 target specific shRNA Plasmids that encode 19-25 nucleotide (plus hairpin) shRNAs that can be used to knock down protein expression in a broad variety of mammalian cell types. Our product line includes shRNA Plasmids designed to silence a large selection of proteins, including tumor suppressors, transcription regulators, cell cycle proteins, membrane receptors, signaling intermediates, kinases, cell adhesion proteins and proteins involved in lymphocyte signaling. In addition, for each shRNA Plasmid DNA product, we offer an appropriate "matched" control antibody for confirmation of targeted mRNA silencing by either Western blotting or fluorescence antibody cell staining. We also offer shRNA Plasmid Transfection Reagent, appropriate buffers and non-targeted control shRNA Plasmids. Santa Cruz Biotechnology, Inc.'s shRNA plasmids are encoded with a puromycin resistance gene for the purpose of isolating shRNA plasmid DNA transfected cells.

PRODUCT

Control shRNA Plasmid-B is a negative control for experiments using targeted shRNA Plasmid DNA transfection; Control shRNA Plasmid-B encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA. After transfection, cells stably expressing the control shRNA may be isolated via puromycin selection. Each vial contains 20 µg lyophilized shRNA plasmid DNA sufficient for up to 20 transfections when resuspended as directed below.

PROTOCOLS

See our web site at www.scbt.com for detailed protocols and support products.

STORAGE AND RESUSPENSION

Store lyophilized shRNA plasmid DNA at 4° C with desiccant. Stable for at least one year from the date of shipment. Once resuspended, store at 4° C for short term storage or -20° C for long term storage. Avoid repeated freeze thaw cycles.

Resuspend lyophilized shRNA Plasmid DNA in 200 µl of the deionized water provided. Resuspension of the shRNA Plasmid DNA in 200 µl of deionized water makes a 0.1 µg/µl solution in a 10 mM Tris, 1 mM EDTA buffered solution.

SUPPORT REAGENTS

PRODUCT	CAT. #	DESCRIPTION	AMOUNT
shRNA Plasmid Transfection Reagent	sc-108061	Delivers shRNA Plasmid DNA into cells with minimal cell toxicity; enables highly efficient shRNA Plasmid DNA transfection in a variety of cell lines including CHO-K1, COS, LNCaP, NIH/3T3, 293, T24, C2C12, SF-9, primary human keratiocytes, primary aortic smoothmuscle, primary rabbit myoblasts, human bone marrow endothelial cells (HBMEC).	0.2 ml 50-100 transfections
shRNA Plasmid Transfection Medium	sc-108062	Reduced-serum medium suitable for addition to shRNA suspension and shRNA Transfection Reagent immediately prior to cell transfection; modification of Eagle's Minimal Essential Medium, buffered with HEPES and sodium bicarbonate, and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements, growth factors and phenol red.	20 ml
Control shRNA Plasmid-A	sc-108060	Control shRNA Plasmid-A is a negative control for experiments using targeted shRNA transfection which encodes a scrambled shRNA sequence that will not lead to the specific degradation of any known cellular mRNA.	20 µg 20 transfections
Control shRNA Plasmid-B	sc-108065	Control shRNA Plasmid-B is available as an alternate negative scrambled shRNA sequence control.	20 µg 20 transfections
Control shRNA Plasmid-C	sc-108066	Control shRNA Plasmid-C is available as an alternate negative scrambled shRNA sequence control.	20 µg 20 transfections

shRNA Plasmid support reagents are optimal for successful delivery of Santa Cruz Biotechnology, Inc.'s shRNA Gene Silencing Plasmids into mammalian cells. Amounts listed above are based on use of 6-well plates.

RESEARCH USE

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