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SANTA CRUZ BIOTECHNOLOGY, INC.

GFP shRNA *(A. vic)* Lentiviral Particles: sc-45924-V

BACKGROUND

The green fluorescent protein (GFP) was originally identified as a protein involved in the bioluminescence of the jellyfish Aequorea victoria. GFP cDNA produces a fluorescent product when expressed in prokaryotic cells, without the need for exogenous substrates or cofactors, making GFP a useful tool for monitoring gene expression and protein localization *in vivo*. Several GFP mutants have been developed, including EGFP, which fluoresce more intensely than the wildtype GFP and have shifted excitation maxima, making them useful for FACS and fluorescence microscopy as well as double-labeling applications. GFP is widely used in expression vectors as a fusion protein tag, allowing expression and monitoring of heterologous proteins fused to GFP.

REFERENCES

1. Prasher, D.C., et al. 1992. Primary structure of the *Aequorea victoria* green fluorescent protein. Gene 111: 229-233.
2. Chalfie, M., et al. 1994. Green fluorescent protein as a marker for gene expression. Science 263: 802-805.
3. Inouye, S., et al. 1994. *Aequorea* green fluorescent protein. Expression of the gene and fluorescence characteristics of the recombinant protein. FEBS Lett. 341: 277-280.
4. Cormack, B.P., et al. 1996. FACS-opitmized mutants of the green fluorescent protein (GFP). Gene 173: 33-38.
5. Rizzuto, R., et al. 1996. Double labelling of the subcellular structures with organelle-targeted GFP mutants *in vivo*. Curr. Biol. 6: 183-188.
6. Enoki, S., et al. 2004. Acid denaturation and refolding of green fluorescent protein. Biochemistry 43: 14238-14248.
7. Lehtinen, J., et al. 2004. Green fluorescent protein-propidium iodide (GFP-PI) based assay for flow cytometric measurement of bacterial viability. Cytometry 60A: 165-172.
8. Gorokhovatsky, et al. 2004. Fusion of *Aequorea victoria* GFP and Aequorin provides their Ca^{2+}-induced interaction that results in red shift of GFP absorption and efficient bioluminescence energy transfer. Biochem. Biophys. Res. Commun. 320: 703-711.

PRODUCT

GFP shRNA *(A. vic)* 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 10^6 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 GFP siRNA *(A. vic):* sc-45924 and GFP shRNA Plasmid *(A. vic):* sc-45924-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

GFP shRNA Plasmid *(A. vic)* is recommended for the inhibition of GFP expression in *Aequorea victoria* cells.

SUPPORT REAGENTS

Control shRNA Lentiviral Particles: sc-108080. Available as 200 µl frozen viral stock containing 1.0 x 10^6 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

GFP (B-2): sc-9996 is recommended as a control antibody for monitoring of GFP 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 GFP gene expression knockdown using RT-PCR Primer: GFP *(A. vic)*-PR: sc-45924-PR (20 µl, 444 bp). 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.

PROTOCOLS

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