SANTA CRUZ BIOTECHNOLOGY, INC.

Trk C Lentiviral Activation Particles (h): sc-400854-LAC

BACKGROUND

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas9) system is an adaptive immune response defense mechanism used by archea and bacteria for the degradation of foreign genetic material. This mechanism can be repurposed for other functions, including genomic engineering for mammalian systems, such as gene knockout (KO) (1,2) and gene activation (3-5). CRISPR Activation Plasmid products enable the identification and upregulation of specific genes by utilizing a D10A and N863A deactivated Cas9 (dCas9) nuclease fused to a VP64 activation domain, in conjunction with sgRNA (MS2), a target-specific sgRNA engineered to bind the MS2-P65-HSF1 fusion protein (5). This synergistic activation mediator (SAM) transcription activation system* provides a robust system to maximize the activation of endogenous gene expression (5).

REFERENCES

1. Cong, L., et al. 2013. Multiplex genome engineering using CRISPR/Cas systems. Science 339: 819-823.
2. Mali, P., et al. 2013. RNA-guided human genome engineering via Cas9. Science 339: 823-826.
3. Maeder, M.L., et al. 2013. CRISPR RNA-guided activation of endogenous human genes. Nat. Methods 10: 977-979.
4. Hsu, P.D., et al. 2014. Development and applications of CRISPR-Cas9 for genome editing. Cell 157: 1262-1278.
5. Konermann, S., et al. 2015. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature 517: 583-588.

CHROMOSOMAL LOCATION

Genetic locus: NTRK3 (human) mapping to 15q25.3.

PRODUCT

Trk C Lentiviral Activation Particles (h) and Trk C Lentiviral Activation Particles (h2) are each a SAM transcription activation system designed to specifically upregulate expression of the NTRK3 (human) gene via lentiviral transduction.

Trk C Lentiviral Activation Particles (h) and Trk C Lentiviral Activation Particles (h2) each contain the following SAM activation elements: a deactivated Cas9 (dCas9) nuclease (D10A and N863A) fused to the transactivation domain VP64, an MS2-p65-HSF1 fusion protein, and a target-specific 20 nt guide RNA. They also contain blasticidin, hygromycin and puromycin resistance genes. The Trk C Lentiviral Activation Particles (h) and Trk C Lentiviral Activation Particles (h2) each encode their own, unique, target-specific 20 nt guide RNA. The resulting SAM complex provides a robust transcription activation system for the upregulation of NTRK3 (human). Each vial contains Lentiviral Activation Particles supplied frozen in 200 µl of Dulbecco's Modified Eagle Medium with 25 mM HEPES pH 7.3, containing enough virus for 10-20 transductions.

PROTOCOLS

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

APPLICATIONS

Either Trk C Lentiviral Activation Particles (h) or Trk C Lentiviral Activation Particles (h2) is recommended for gene activation in human cells.

SUPPORT REAGENTS

For optimal reaction efficiency with Lentiviral Activation Particle products, Polybrene®: sc-134220 (10 mg/ml) is recommended. Hygromycin B solution: sc-29067 (1 g), Blasticidin S HCl solution: sc-495389 (1 ml) and Puromycin dihydrochloride: sc-108071 (25 mg) are recommended for selection. Control Lentiviral Activation Particles: sc-437282 (200 µl) negative control is also available.

GENE EXPRESSION MONITORING

Trk C (7H6): sc-517245 is recommended as a control antibody for monitoring of NTRK3 (human) gene expression prior to and after activation 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).

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 SAM components into genomic DNA of target cells.

STORAGE

Store Lentiviral Activation 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.

RESEARCH USE

The Lentiviral Activation Particle products are considered "Licensed Products" and are to be used in accordance with the Limited License stated on www.scbt.com/limitedlicense. 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.