SANTA CRUZ BIOTECHNOLOGY, INC.

Rab 10 CRISPR Activation Plasmid (m): sc-422551-ACT

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 (3-6). 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 (6). This synergistic activation mediator (SAM) transcription activation system provides a robust system to maximize the activation of endogenous gene expression (6).

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. Van der Oost, J., et al. 2014. Unraveling the structural and mechanistic basis of CRISPR-Cas systems. Nat. Rev. Microbiol. 12: 479-492.
6. Konermann, S., et al. 2015. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. Nature 517: 583-588.

CHROMOSOMAL LOCATION

Genetic locus: Rab10 (mouse) mapping to 12 A1.1.

PRODUCT

Rab 10 CRISPR Activation Plasmid (m) and Rab 10 CRISPR Activation Plasmid (m2) are each a SAM transcription activation system designed to specifically upregulate expression of the Rab10 (mouse) gene.

Rab 10 CRISPR Activation Plasmid (m) and Rab 10 CRISPR Activation Plasmid (m2) each consist of the following 3 plasmids at a 1:1:1 mass ratio: the CRISPR/dCas9-VP64-Blast plasmid encoding the deactivated Cas9 (dCas9) nuclease (D10A and N863A) fused to the transactivation domain VP64, and a blasticidin resistance gene; the MS2-P65-HSF1-Hygro plasmid encoding the MS2-p65-HSF1 fusion protein, and a hygromycin resistance gene; the sgRNA (MS2)-Puro plasmid encoding a target-specific 20 nt guide RNA, and a puromycin resistance gene. The sgRNA (MS2)-Puro plasmids in Rab 10 CRISPR Activation Plasmid (m) and Rab 10 CRISPR Activation Plasmid (m2) 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 Rab10 (mouse). Each vial contains 20 µg of lyophilized CRISPR Activation Plasmid DNA. Suitable for up to 20 transfections.

APPLICATIONS

Either Rab 10 CRISPR Activation Plasmid (m) or Rab 10 CRISPR Activation Plasmid (m2) is recommended to increase activation of gene expression in mouse cells.

SUPPORT REAGENTS

For optimal reaction efficiency with CRISPR Activation Plasmids, Santa Cruz Biotechnology's UltraCruz® Transfection Reagent: sc-395739 (0.2 ml) and Plasmid Transfection Medium: sc-108062 (20 ml) are 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 CRISPR Activation Plasmid: sc-437275 (20 µg) negative control is also available.

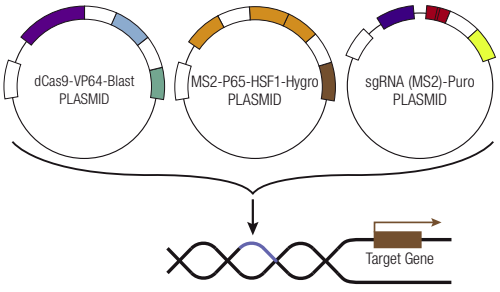
GENE EXPRESSION MONITORING

Rab 10 (2A1C9): sc-101429 is recommended as a control antibody for monitoring of Rab10 (mouse) 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).

STORAGE AND RESUSPENSION

Store lyophilized 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 plasmid DNA in 200 µl of the provided ultrapure, sterile, DNase-free water. Resuspension of the plasmid DNA makes a 0.1 µg/µl solution in a 10 mM TRIS EDTA, 1 mM EDTA buffered solution.

*SAM Transcription Activation System



SAM complex binds to a specific site located upstream of the transcriptional start site (TSS) of the target gene and recruits transcription factors, thereby activating endogenous transcription of the target gene.

RESEARCH USE

The CRISPR Activation Plasmids 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.