TGFβ RII CRISPR/Cas9 KO Plasmid (m2): sc-423371-KO-2

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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 (4,6). This mechanism can be repurposed for other functions, including genomic engineering for mammalian systems, such as gene knockout (KO) (1,2,3,5). CRISPR/Cas9 KO Plasmid products enable the identification and cleavage of specific genes by utilizing guide RNA (gRNA) sequences derived from the Genome-scale CRISPR Knock-Out (GeCKO) v2 library developed in the Zhang Laboratory at the Broad Institute (3,5).

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. Ran, F.A., et al. 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8: 2281-2308.

4. Van der Oost, J., et al. 2014. Unraveling the structural and mechanistic basis of CRISPR-Cas systems. Nat. Rev. Microbiol. 12: 479-492.

5. Shalem, O., et al. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343: 84-87.

6. Hsu, P.D., et al. 2014. Development and applications of CRISPR-Cas9 for genome engineering. Cell 157: 1262-1278.

CHROMOSOMAL LOCATION

Genetic locus: Tgfbr2 (mouse) mapping to 9 F3.

PRODUCT

TGFβ RII CRISPR/Cas9 KO Plasmid (m2) is designed to disrupt gene expression by causing a double-strand break (DSB) in a 5' constitutive exon within the Tgfbr2 (mouse) gene.

TGFβ RII CRISPR/Cas9 KO Plasmid (m2) consists of a pool of 3 plasmids, each encoding the Cas9 nuclease and a target-specific 20 nt guide RNA (gRNA) designed for maximum knockout efficiency. Each vial contains 20 µg of lyophilized CRISPR/Cas9 Plasmid DNA. Suitable for up to 20 transfections. Also see TGFβ RII HDR Plasmid (m2): sc-423371-HDR-2 for selection of cells containing a DSB induced by TGFβ RII CRISPR/Cas9 KO Plasmid (m2).

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.

APPLICATIONS

TGFβ RII CRISPR/Cas9 KO Plasmid (m2) is recommended for the disruption of gene expression in mouse cells.
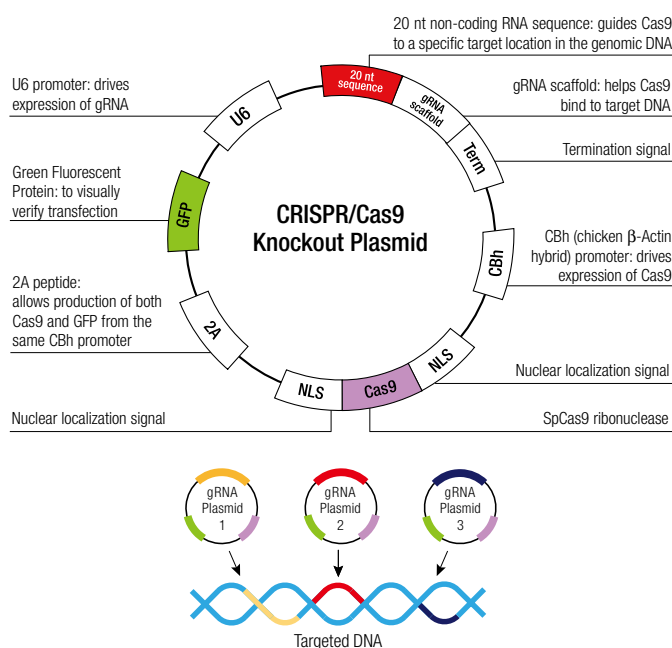
SUPPORT REAGENTS

For optimal reaction efficiency with CRISPR/Cas9 KO Plasmids, Santa Cruz Biotechnology's UltraCruz® Transfection Reagent: sc-395739 (0.2 ml) and Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control CRISPR/Cas9 Plasmid: sc-418922 (20 µg) negative control is also available.

GENE EXPRESSION MONITORING

TGFβ RII (E-6): sc-17792 isrecommended as control antibody for monitoring of Tgfbr2 (mouse) gene expression prior to and after knockout 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).

RESEARCH USE

The CRISPR/Cas9 KO Plasmids are considered "Licensed Products" and are to be used in accordance with the Limited License stated on www.scbt.com/limitedlicense.

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