GAP1-InsP₄ BP CRISPR/Cas9 KO Plasmid (h): sc-403002



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

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- Ran, F.A., et al. 2013. Genome engineering using the CRISPR-Cas9 system. Nat. Protoc. 8: 2281-2308.
- Van der Oost, J., et al. 2014. Unraveling the structural and mechanistic basis of CRISPR-Cas systems. Nat. Rev. Microbiol. 12: 479-492.
- Shalem, O., et al. 2014. Genome-scale CRISPR-Cas9 knockout screening in human cells. Science 343: 84-87.
- Hsu, P.D., et al. 2014. Development and applications of CRISPR-Cas9 for genome editing. Cell 157: 1262-1278.

CHROMOSOMAL LOCATION

Genetic locus: RASA3 (human) mapping to 13q34.

PRODUCT

 ${\rm GAP1\text{-}InsP_4}$ BP CRISPR/Cas9 KO Plasmid (h) is designed to disrupt gene expression by causing a double-strand break (DSB) in a 5' constitutive exon within the RASA3 (human) gene.

GAP1-InsP4 BP CRISPR/Cas9 KO Plasmid (h) 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 μ g of lyophilized CRISPR/Cas9 Plasmid DNA. Suitable for up to 20 transfections. Also see GAP1-InsP4 BP HDR Plasmid (h): sc-403002-HDR for selection of cells containing a DSB induced by GAP1-InsP4 BP CRISPR/Cas9 KO Plasmid (h).

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 μ l of the provided ultrapure, sterile, DNase-free water. Resuspension of the plasmid DNA makes a 0.1 μ g/ μ l solution in a 10 mM TRIS EDTA, 1 mM EDTA buffered solution.

APPLICATIONS

 ${\sf GAP1-InsP_4}$ BP CRISPR/Cas9 KO Plasmid (h) is recommended for the disruption of gene expression in human cells.

20 nt non-coding RNA sequence: guides Cas9 to a specific target location in the genomic DNA U6 promoter: drives gRNA scaffold: helps Cas9 expression of gRNA bind to target DNA Termination signal Green Fluorescent Protein: to visually verify transfection CRISPR/Cas9 CBh (chicken B-Actin **Knockout Plasmid** hybrid) promoter: drives expression of Cas9 2A peptide: allows production of both Cas9 and GFP from the same CBh promoter Nuclear localization signal NLS Nuclear localization signal SpCas9 ribonuclease Targeted DNA

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

GAP1-InsP₄ BP (E-9): sc-398283 is recommended as a control antibody for monitoring of RASA3 (human) 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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