

cystatin D Double Nickase Plasmid (h): sc-405049-NIC

BACKGROUND

The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas9) system is an adaptive immune response defense mechanism used by archaea and bacteria for the degradation of foreign genetic material (6). This mechanism can be repurposed for other functions, including genomic engineering for mammalian systems, such as gene knockout (KO) (1,2,3). CRISPR/Cas9 KO Plasmid products enable the identification and cleavage of specific genes by utilizing guide RNA (gRNA) sequences. While the CRISPR/Cas9 KO Plasmids enable maximum gene knockout efficiency, CRISPR Double Nickase Plasmid products offer improved specificity while maintaining a high level of knockout efficiency (4).

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. Ran, F.A., et al. 2013. Double nicking by RNA-guided CRISPR Cas9 for enhanced genome editing specificity. *Cell* 154: 1380-1389.
5. Hsu, P.D., et al. 2014. Development and applications of CRISPR-Cas9 for genome editing. *Cell* 157: 1262-1278.

CHROMOSOMAL LOCATION

Genetic locus: CST5 (human) mapping to 20p11.21.

PRODUCT

cystatin D Double Nickase Plasmid (h) and cystatin D Double Nickase Plasmid (h2) designed to disrupt gene expression by causing highly specific Cas9-mediated double nicking of the CST5 (human) gene, which mimics a double-strand break (DSB).

cystatin D Double Nickase Plasmid (h) and cystatin D Double Nickase Plasmid (h2) each consist of a pair of plasmids, each encoding a D10A mutated Cas9 nuclease and a unique, target-specific 20 nt guide RNA (gRNA). Each pair of gRNA sequences are offset by approximately 20 bp to allow for gene knockout with greater specificity than their CRISPR/Cas9 KO Plasmid counterpart. One plasmid in the pair contains a puromycin resistance gene for selection; the other plasmid in the pair contains a GFP marker to visually confirm transfection. Each vial contains 20 µg of lyophilized Double Nickase Plasmid DNA. Suitable for up to 20 transfections.

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

Either cystatin D Double Nickase Plasmid (h) or cystatin D Double Nickase Plasmid (h2) is recommended for the disruption of gene expression in human cells.



SUPPORT REAGENTS

For optimal reaction efficiency with Double Nickase Plasmids, Santa Cruz Biotechnology's UltraCruz[®] Transfection Reagent: sc-395739 (0.2 ml) and Plasmid Transfection Medium: sc-108062 (20 ml) are recommended. Control Double Nickase Plasmid: sc-437281 (20 µg) negative control is also available.

GENE EXPRESSION MONITORING

cystatin D (F-3): sc-398553 is recommended as a control antibody for monitoring of CST5 (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).

RESEARCH USE

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