SANTA CRUZ BIOTECHNOLOGY, INC.

Insulin Double Nickase Plasmid (h): sc-400149-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 archea 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: INS (human) mapping to 11p15.5.

PRODUCT

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

Insulin Double Nickase Plasmid (h) and Insulin 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.

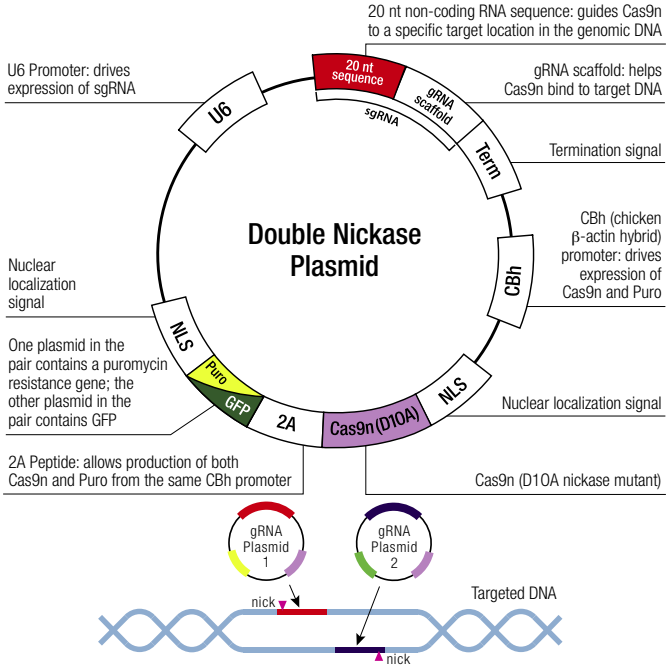
STORAGE AND RESUSPENSION

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 Insulin Double Nickase Plasmid (h) or Insulin 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

Insulin (2D11-H5): sc-8033 is recommended as a control antibody for monitoring of INS (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

The Double Nickase 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.