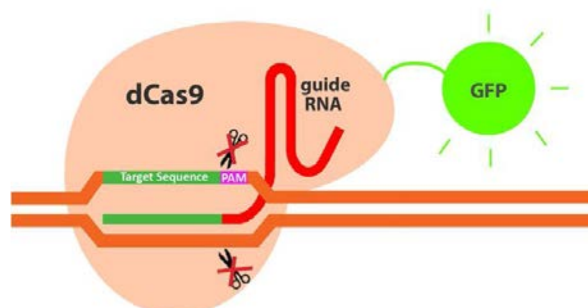
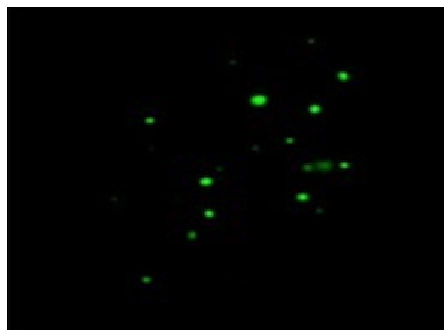
NOVATEINBIO
Accelerates Research and Development

NLS-dCas9-EGFP-NLS, Cat# PR-137213G





Background	NLS-dCas9-EGFP is a fusion protein which has both nuclear localization sequence (NLS) on its N terminal and EGFP-NLS on the C terminal. The dCas9 RNP complex can localize to the nucleus immediately upon entering the cell. The EGFP can be taken as a reporter for tracking or sorting transfected cells, which creates the possibility of enriching cell populations for desired genome edits via fluorescence activated cell sorting (FACS). This fusion protein can also be used to do subcellular localization of certain specific sequence. NLS-dCas9-EGFP is better than NLS-Cas9-EGFP in genome tracking by in situ hybridization, due to the inactivity of nuclease while retaining the DNA binding ability.	
Concentration	50 µg/ 50 µl (280 pmol), 5.31 µM.	
Source	*E. coli*	
Buffer	10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol	
Appearance	Sterile filtered colorless solution. Or Powder	
Formulation	Recombinant NLS-dCas9-EGFP-NLS protein expressed in E. coli supplied in a buffer of 10 mM Tris-HCl (pH 7.4), 0.1 mM EDTA, 1 mM DTT, 300 mM NaCl, and 50% (v/v) Glycerol. **For dry powder, please add 250 µL nuclease-free water with 50% (v/v) Glycerol. Incubate on ice for > 20 min. Then mix well. The concentration is 0.2 µg/ µl , or 1.06 µM.**	
Storage	-70°C. Avoid freeze thaw cycles.	
10X Reaction Buffer	Concentration	10X (1 ml)
	Buffer	20 mM HEPES, 100 mM NaCl, 5 mM MgCl2, 0.1 mM EDTA, pH 6.5
	Storage	-20 °C

RNP Complex Formation

Gently mix the reaction and incubate at room temperature for 10 minutes.
Then place on ice for following transfection by electroporation or liposome.

Components	Volume	Final Concentration
sgRNA (1000 nM)	1.2 µl	~120 nM
Cas9 Nuclease Protein (1000 nM)	1.2 µl	~120 nM
Opti-MEM	12.6 µl	-
Total	15 µl	

Trypsinize and Prepare HEK293T Cells

1. Seed the cells so that they will be around 70-90% confluent on the day of transfection.
2. During the RNP/liposome incubation, trypsinize the cells, washing once to remove any traces Of trypsin. Resuspend the cells in 10 ml of media and count.
3. Calculate the dilution and volume needed to get the cells to 3.2×10^5 cells per ml. You will need 125 µl of cells per well in 96-well plate.

Transfect Cells with Liposome Complexes

1. From each tube of RNP/liposome complex, aliquot 25 µl into 3 wells of a 96-well plate.
2. Add 125 µl of cells (3.2×10^5 cells/ml) to each well containing RNP/liposome complex and pipette up and down gently a few times.
3. Incubate the cells in a humidified 37°C, 5% CO_2 incubator for 48-72 hours.

Quality Control:

Exonuclease, endonuclease and non-specific nuclease activities not detected.