Product Analysis Certificate

PRODUCT	**Recombinant Cas9 protein (derived from *Streptococcus pyogenes) with NLS***
CATALOG #	**CAS400A-1**
LOT #	**PCxxxx**
APPEARANCE	**White powder**
MW	**163 kD**
STORAGE	**-80°C (long term), -20°C (short term)**
SHELF LIFE	**12 months from date of receipt with proper storage**
SHIPPING	**Dry ice**

DESCRIPTION

SBI's highly purified recombinant Cas9 protein is designed to combine with in vitro transcribed guide RNA for more efficient and precise genome editing in cells while reducing off-target effects and avoiding unwanted integration of plasmid DNA in the host genome. The recombinant Cas9 protein was isolated from an *E. coli* host carrying cloned Cas9 cDNA from *S. pyogenes* with 6x His tag and SV40 nuclear localization signal. The purified protein may be utilized for a wide range of assays in target cells, including but not limited to:

- Transfection of cells *in vitro*
- Embryo injection
- *In vitro* cleavage assay

PACKAGE CONTENTS

Cat#	Description	Amount
CAS400A-1	Recombinant Cas9 protein with 6x His tag and nuclear localization signal (NLS) from SV40; Reconstitutes to 1 µg/µl (~6 µM)	**50 µg/vial**

HANDLING GUIDELINES

- Store lyophilized product at -20°C for short term or -80°C for long term (>1 month).

- To reconstitute, add 50 µl nuclease free water. For embryo injection, use water for reconstitution and aliquot for a single use. For other applications, having glycerol helps the protein stability during freeze and thaw. Cas9 protein can go through at least three rounds of freeze and thaw cycle without losing the activity significantly.

- The final reconstituted product is 1 µg/µl in 20 mM HEPES, 150 mM KCl, and 1% sucrose (pH 7.5).

- For applications requiring higher concentration, use half volume for reconstitution to make 2 µg/µl stock.

- If lower concentration is desired, use dilution buffer (20 mM HEPES, 150 mM KCl, 1% sucrose, pH 7.5). Alternatively, 1x NEB buffer 3 can be used for short-term storage

- Mix well by pipetting and vortex.

- Store aliquots at – 80°C. For short term (about a month), -20°C can be used.

PROTOCOLS:

A) IN VITRO CLEAVAGE ASSAY

1. Set up the reaction mixture as below. Add Cas9 protein and sgRNA last for best results.

Components	Amount	Range
Cas9 protein	150 ng	50-200 ng
sgRNA	100 ng	30-200 ng
Target DNA	60 ng PCR product	50-100 ng
	or 100 ng plasmid	50-200 ng
10x NEB buffer 3	1 μl	
10x BSA	1 μl	
Nuclease free water	10 μl	

2. Incubate the reaction mixture at 37°C for 1 hr.

3. Heat to inactivate Cas9 protein at 65°C for 10 min.

4. Analyze on agarose gel.

5. In rare cases, Cas9/sgRNA complex is still bound to DNA template and result in abnormal migration. If it is the case, add 4 μg of RNase and incubate for 15 min at 37 °C, followed by addition of 1 μl of STOP solution (30% glycerol, 1% SDS, 250 mM EDTA, pH 8.0) to the reaction mixture. Incubate for 15 min at 37 °C before gel running.

B) PROTOCOL FOR TRANSFECTION OF CAS9 PROTEIN AND GUIDE RNA

1) Plate 100,000 to 200,000 of target cells (e.g. 293T cells) into a single well of a 12-well plate in 1 ml of appropriate growth medium. Include a single well of cells as negative control.

2) Next day, or when cells are 50-60% confluent, transfect target cells with the Cas9 protein and gRNA (and appropriate donor vector if HDR is desired) using a suitable transfection reagent (e.g. Lipofectamine 2000/3000) following the manufacturer's recommended protocol for 12-well plates. The use of reduced or serum-free media (e.g. OptiMEM) containing no antibiotics to dilute the vector/transfection complex is highly recommended.

Note: Purified Cas9 protein and sgRNA at molar ratios (1:1) with 0.5 μg HR donor vector was used in EGIP 293T cells for HDR application (see data below). For other cell lines, we suggest optimizing the amounts and ratios of Cas9 protein, gRNA, and donor vector for optimal results.

3) Allow at least 12 hours before changing transfection media to complete growth media

4) 48-96 hours after initial transfection, assay for cleavage activity using Surveyor Nuclease, PCR genotyping analysis, or HDR activity (if using donor vector in parallel)

VALIDATION DATA:

Cas9 Plasmid **Cas9 Protein**

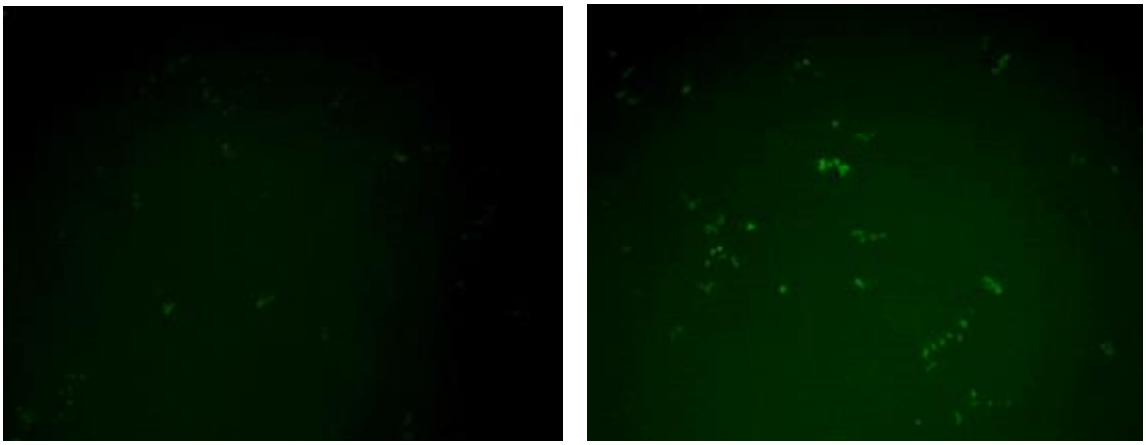

Fig 1. HDR rescue efficiency of donor EGFP fragment for Cas9 protein + gRNA system (right) compared with all-in-one Cas9 plasmid system (left) as measured by GFP positive clones at day 3 post-transfection into cell line with mutant EGFP gene (EGIP).

Important Licensing Information

SBI has pending patent applications related to the Cas9 SmartNuclease Products. SBI does not require a use-license for research purposes for non-profit or for-profit institutions. For commercial applications, please contact SBI for more information. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use. This Product is covered by a limited-use label license. Purchase and use of any part of the Product constitutes acceptance of the terms outlined in the Licensing and Warranty Statement contained in the User Manual. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI).