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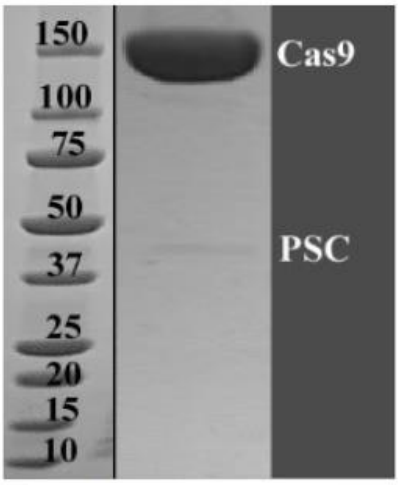
Data Sheet

Purified CRISPR-Cas9 Protein

SKU No.: 40-1020, 40-1025

Description	Cas9 protein combines with guide RNA (gRNA) to form a stable ribonucleoprotein (RNP) complex that enables efficient and precise genome editing in cells.
Purity	>99% (SDS gel analysis)
Enzyme Source	*Streptococcus pyogenes* Cas9 is expressed in *E. coli* with two (N-terminal and C-terminal) nuclear localization signals (NLS) and is purified with our CL7 tag technology.
Storage Buffer	10 mM Tris-Cl pH 8.0, 250 mM NaCl, 50% glycerol
Storage/Shipping Concentration	~5 mg/mL
Shipping Conditions	Dry ice
Recommended Storage Conditions	-80°C
Endotoxin	<0.3 EU/µg by rFC method (Arvys Proteins Inc.)

Product Data



~99% Purity

Figure 1. Purification of Cas9 with CL7 tag system results in ~99% purity. PSC is PreScission protease left over from cleavage.

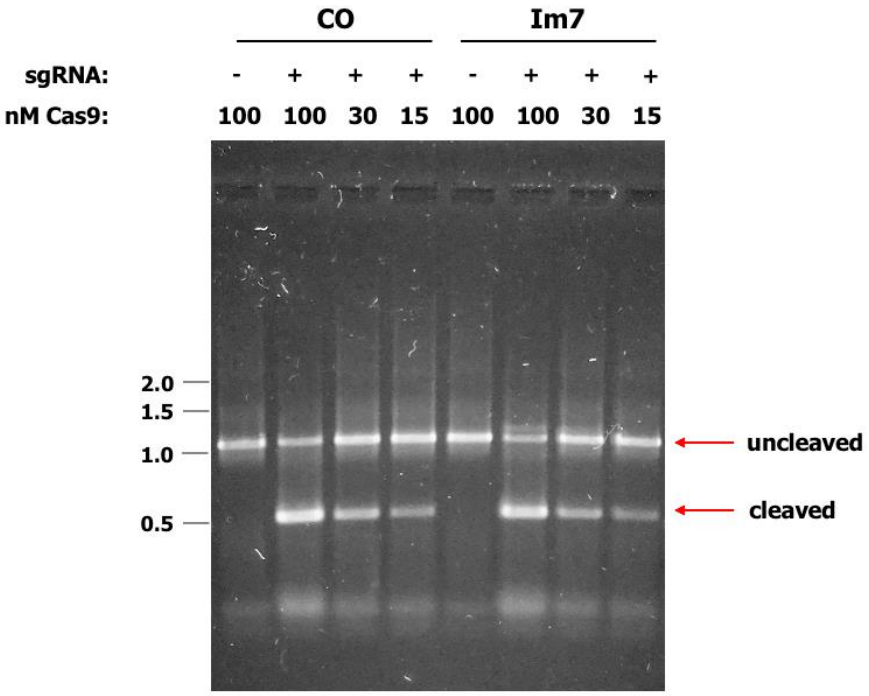
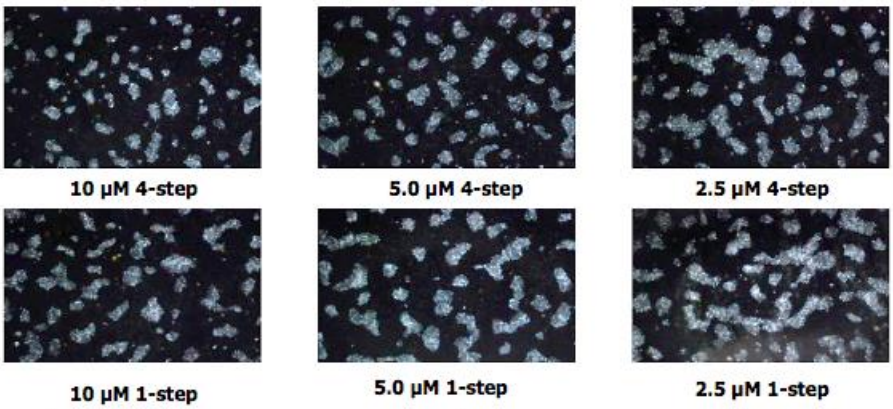


Figure 2. CL7/Im7-purified-Cas9's cleavage activity compared to commercially obtained Cas9. Reactions were resolved on a 1.2% agarose/TAE gel which was then stained with SYBR GOLD.

Cas9 obtained from a commercial source (CO) or CL7/Im7-purified Cas9 (Im7) was complexed with equimolar (+), or without (-), an annealed sgRNA. Complexes were incubated at different concentrations with a 1.1 kb target DNA fragment for 15 minutes at 37°C. The sgRNA recognizes a sequence in the middle of the target DNA, resulting in ~550 bp fragments upon cleavage. Im7-purified Cas9 showed comparable activity to commercial Cas9.

A



B

	RNP Conc.	% Viability vs. buffer electroporation	% Viability vs. no electroporation	HDR	Total modification
Cas9WT 4-step	10 µM	55%	17.6%	51.0%	89.0%
	5.0 µM	67%	21.8%	41.9%	83.3%
	2.5 µM	64%	20.6%	23.3%	73.6%
Cas9WT 1-step	10 µM	64%	20.6%	50.9%	88.2%
	5.0 µM	64%	20.6%	45.1%	90.1%
	2.5 µM	73%	23.5%	22.4%	74.3%

Figure 2. Activities of CL7/Im7-purified Cas9 and 4-step purified Cas9 are the same in cells.

(A) Phase contrast microscopy of human sickle cell iPSCs (induced pluripotent stem cells) after electroporation with Lonza nucleofector 2b and varying concentrations of RNP using Cas9WT from either the 4-step purification (top row) or 1-step purification protocols (bottom row).

(B) Table of viabilities and modifications from the electroporation experiments shown in **(A)**. RNPs made with Cas9 purified using the two protocols were tested using a sgRNA designed to correct a 1-bp error in hemoglobin that causes sickle cell disease. Modification rates were evaluated by digital PCR. The proteins resulted in the same viability and modification rates, indicating equivalent efficiency in cells. These tests were performed by Tim Townes and Lei Ding of the University of Alabama at Birmingham (UAB).

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