Recombinant *S. pyogenes* CRISPR-Cas9

Catalog Number: 9957-C9

DESCRIPTION

Source

E. coli-derived *s. pyogenes* CRISPR-Cas9 protein

APKKKRKVGIHGVPAA	*S. pyogenes* CRISPR-Cas9 (Asp2-Asp1368) Accession # Q99ZW2	KRPAATKKAGQAKK-KKGYGRKKRRQRRRG	HHHHHH

N-terminus C-terminus

N-terminal Sequence Analysis Ala

Predicted Molecular Mass 164 kDa

SPECIFICATIONS

SDS-PAGE 133 kDa, reducing conditions

Activity Measured by its ability to cleave a targeted DNA substrate.
S. pyogenes CRISPR-Cas9 achieves >80% substrate cleavage, as measured under the described conditions.

Endotoxin Level <0.10 EU per 1 µg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 µm filtered solution in Tris, NaCl, EDTA, Glycerol and TCEP. See Certificate of Analysis for details.

Activity Assay Protocol

Materials

- Assay Buffer: 50 mM NaCl, 10 mM Tris-HCl, 10 mM $MgCl_2$, 100 µg/mL BSA, pH 7.9
- Recombinant *Streptococcus pyogenes* CRISPR-Cas9 (r*S. pyogenes* Cas9) (Catalog # 9957-C9)
- PBR322 vector (NEB, Catalog # N3033S) digested with EcoRI-HF (NEB, Catalog # R3101S)*
- Dharmacon synthetic sgRNA, targeting sequence: GAGGCAGACAAGGTATAGGG
- Ethidium Bromide, 10 mg/mL (Amresco, Catalog # X328)
- Ultrapure DNase/RNase-Free Distilled Water (Invitrogen, Catalog # 10977015), to prepare Assay Buffer
- DNA gel

*Digest was gel purified using gel purification kit and eluted in EB buffer (10 mM Tris-HCl, pH 8.5).

Assay

1. Prepare RNP Complex:
   a. 600 nM sgRNA (6 µL addition from 3 µM stock prepared in Assay Buffer)
   b. 0.25 µg r*S. pyogenes* Cas9
   c. Add Assay Buffer for a final RNP Complex volume of 26.5 µL
   d. Incubate for 5 minutes at 37 °C.
2. Mix RNP Complex with 3.5 µL of 8.6 ng/µL of DNA Substrate (diluted in Assay Buffer, if possible).
3. Incubate for 1 hour at 37 °C.
4. Incubate for 10 minutes at 65 °C to dissociate enzyme from DNA.
5. Load total reaction with loading dye on a 1% agarose gel.
6. Run gel at 140 V for 40 minutes.
7. Soak gel in 200 mL TAE with 150 µL of 10 mg/mL ethidium bromide for 1 hour.
8. Use imaging software to detect and quantify hydrolysis of the DNA substrate.

Final Assay Conditions

Per Reaction:

- r*S. pyogenes* Cas9: 0.25 µg
- DNA Substrate: 30 ng
- sgRNA: 600 nM

PREPARATION AND STORAGE

Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage **Use a manual defrost freezer and avoid repeated freeze-thaw cycles.**

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA

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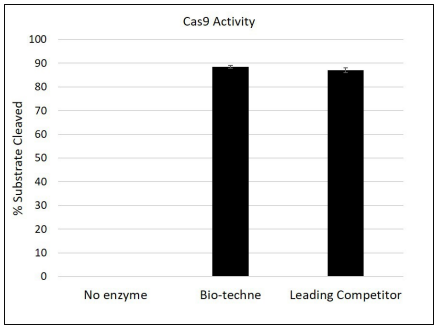
Recombinant *S. pyogenes* CRISPR-Cas9

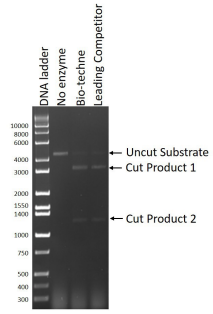
Catalog Number: 9957-C9

Bioactivity



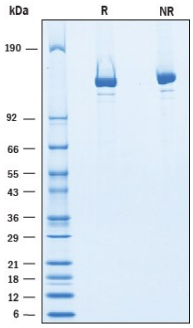
Quantitative analysis of the substrate cleavage by densitometry of the agarose gel. Error bars display standard error of 3 replicates. 9957-C9 has equivalent activity to a Leading Competitor's Cas9 using the insert assay protocol.

Bioactivity



Agarose gel image of in vitro cleavage of DNA substrate into two DNA products by 9957-C9 and a Leading Competitor's Cas9.

SDS-PAGE



2 µg/lane of Recombinant S. pyogenes CRISPR-Cas9 was resolved with SDS-PAGE under reducing (R) and non-reducing (NR) conditions and visualized by Coomassie® Blue staining, showing a band at ~130 kDa.

BACKGROUND

Streptococcus pyogenes Cas9 (CRISPR associated protein 9) is a 160 kDa RNA guided endonuclease that introduces site specific cleavage of double strand DNA (1). It is part of the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) system found in many bacteria such as *S. pyogenes* and most archaea, which provide adaptive immunity against invading mobile genetic elements (such as viruses, transposable elements and conjugative plasmids) (2, 3). Upon viral infection, short viral DNA (known as "spacers") integrate into the host genome between CRISPR repeats, and RNA sequences (guide RNA or gRNA) with this genetic information help guide Cas9 protein to recognize and cut foreign DNA. Cas9 protein undergoes conformational changes upon gRNA binding that shift from non-DNA binding conformation into an active DNA binding conformation. In the Cas9-gRNA complex, the gRNA sequence remains accessible to interact with free DNA, and the extent to which the gRNA spacer and target DNA segment (known as "protospacer") match will determine the cut site (4). The presence of a 5′-NGG-3′ protospacer adjacent motif (PAM) sequence immediately downstream of protospacers is required for Cas9 cleavage of the foreign DNA. PAM is absent in bacterial CRISPR loci, therefore preventing cleavage of the host genome (4). Cas9 associates with other proteins of the acquisition machinery (Cas1, Cas2 and Csn2), presumably to provide PAM specificity to this process (5). This RNA guided nuclease system called CRISPR/Cas (CRISPR associated protein) has been widely applied to genome engineering with increased efficiency (6). The attached nuclear localization signals(NLSs) on the chimeric protein ensures nuclear compartmentalization in mammalian cells during gene editing (7).

References:

1. Feng, Z. *et al.* (2013) Cell **154**:1380.
2. Moineau. *et al.* (2010) Nature **468:**67.
3. Barrangou, R. *et al.* (2014) Molecular Cell **54**:234.
4. Charpentier, E. *et al.* (2011) Nature **471**:602.
5. Heler, R. *et al.* (2015) Nature **519**:199.
6. Thomson, J.A. *et al.* (2013) PNAS,**110**:15644.
7. Cong, L. *et al.* (2013) Science **339**:819.

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