

CS09-E311H-50	50 µg
CS09-E311H-100	100 µg
CS09-E311H-250	250 µg
CS09-E311H-1000	1000 µg

SpCas9, Active

Recombinant *S. pyogenes* protein expressed in *E. coli*

Catalog # CS09-E311H

Lot # T4216-7

Product Description

Recombinant full-length *Streptococcus pyogenes* Cas9 was expressed in *E. coli* with a C-terminal His tag. The protein accession number is [AAK33936](#).

Alternative name (s)

Cas9, Csn1, Spy_1046, Uniprot accession [Q99ZW2](#).

Formulation

Recombinant protein stored in 50mM sodium phosphate, pH 8.0, 300mM NaCl, 150mM imidazole, 1mM DTT, 10% glycerol.

Storage and Stability

Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles.

Always include RNase inhibitor at manufacturer recommended concentrations in Cas enzyme reactions.

Scientific Background

Streptococcus pyogenes Cas9 is a type II-A Cas9 protein and is the most used in most current CRISPR-based genetic engineering methodologies (1). CRISPR RNAs (crRNAs) fused to trans-activating CRISPR RNAs (tracrRNA) direct sequence-specific cleavage by Cas9, introducing double-stranded breaks in cell-invading DNA in bacterial and archaeal CRISPR/Cas9 systems (1,2). Cas9 requires the 5'-NGG-3' protospacer adjacent motif (PAM) to destabilize the target DNA (3). Cas9's HNH endonuclease domain cleaves the strand complementary to the crRNA while its RuvC endonuclease domain cleaves the non-complementary strand (2).

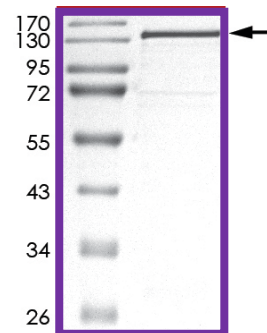
References

1. Jinek, M. et al. 2014. Structures of Cas9 Endonucleases Reveal RNA-Mediated Conformational Activation. *Science*. 343(6176): 1247997.
2. Jinek, M. et al. 2012. A programmable dual RNA-guided endonuclease in adaptive bacterial immunity. *Science*. 337(6096): 816-821.
3. Anders, C. et al. 2014. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. *Nature*. 513(7519): 569-573.

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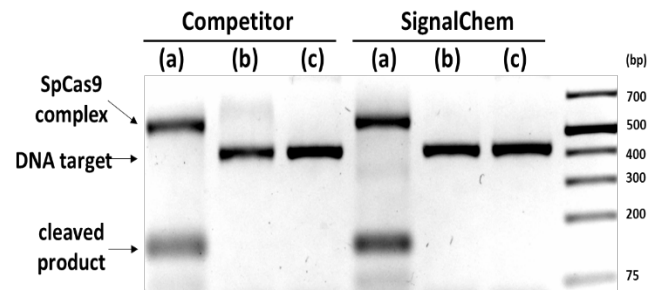
Purity



SDS-PAGE gel image

The purity of SpCas9 was determined to be **>80%** by densitometry.
Observed MW **~156 kDa**
Calculated MW **~160 kDa**

Activity



SpCas9 mediated specific endogenous DNA cleavage. The reactions consist of (a) Target DNA, crRNA, tracrRNA and SpCas9; (b) Target DNA, crRNA and SpCas9; (c) Target DNA and crRNA.

SpCas9, Active

Recombinant *S. pyogenes* protein expressed in *E. coli*

Catalog #	CS09-E311H
Lot #	T4216-7
Purity	>80%
Concentration	0.5 µg/µl 6.26 pmol/µg
Stability	1yr at -70°C from date of shipment
Storage & Shipping	Store product at -70°C . For optimal storage, aliquot target into smaller quantities after centrifugation and store at recommended temperature. For most favorable performance, avoid repeated handling and multiple freeze/thaw cycles. Product shipped on dry ice.

Cas9 Activity Assay Protocol

Reaction Components

Active Cas9 Enzyme

It is recommended that the researcher perform a serial dilution of Active Cas9 for optimal results.

4X Cas9 Reaction Buffer (User Prepared)

200mM Tris-HCl, pH 7.9, 400mM NaCl, 40mM MgCl₂, and 0.4 mg/ml Prionex (w/v 0.04%).

Assay Protocol

Cas9 activity is determined in a DNA cleavage assay. The following conditions may be different for different target DNA sequences. Optimize the protocol for each specific DNA sequence.

Step 1. In an Eppendorf tube, add the following reaction components to generate the Cas9-gRNA complex:

- Component 1.** Active Cas9 enzyme
- Component 2.** Single guide RNA (sgRNA), or a mix of tracrRNA/crRNA
- Component 3.** 5µL of 4X Cas9 Reaction Buffer
- Component 4.** Nuclease-free water

Note: The final reaction volume is 20µL after the addition of the target DNA in Step 3.

After accounting for the volume of target DNA to add (in Step 3), add nuclease-free water to achieve the 20µL final volume.

Step 2. Incubate the above solution at room temperature for 20-60 minutes.

Step 3. Add 50-500ng of target DNA to the Cas9-gRNA complex to initiate the reaction.

Step 4. Incubate the above reaction at 37°C for 10 to 30 minutes.

Step 5. Run the resulting product on a DNA agarose gel to confirm cleavage of the target DNA.

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SAFETY DATA SHEET

Article 1 - Product Identification

Product Name: SpCas9, Active

Catalog # CS09-E311H

This product is sold only for research use by qualified laboratory personnel, and is not to be used as a drug, medical device, food additive, cosmetic, nor household chemical. It is not to be used in diagnostic, therapeutic, consumer, agricultural, nor pesticidal applications.

Manufacturer's Name: SignalChem Biotech Inc.
 Street Address: 110-13120 Vanier Place
 City, Prov. Postal Code: Richmond, BC, V6V 2J2
 Fax: 604-232-4601
 EMERGENCY PHONE: 604-232-4600

Article 2 - Hazard Identification

- **WHMIS Classification:** Not WHMIS controlled.
- **GHS classification:** None.
- **Hazard Pictograms:** None.
- **Signal words:** None.
- **Hazard statements:** None.
- **Precautionary statements:** None.
- **Other hazards:** None known.

Article 3 – Composition/Information on Ingredients

Chemical Characterization: Mixture.

Description: This product consists of the substances listed below.

Common name	Chemical name	CAS-No.	Concentration
Glycerol	Glycerol	56-81-5	10%
NaCl	Sodium chloride	7647-14-5	1.75%
Imidazole	1,3-Diaza-2,4-cyclopentadiene	288-32-4	≤1.02%
Sodium Phosphate, Dibasic	Sodium Phosphate, Dibasic	7782-85-6	1.34%
DTT; Dithiothreitol	(R*,R*)-1,4-Dimercaptobutane-2,3-diol	3483-12-3	0.0154%
Protein	N/A	N/A	≤0.01%

Article 4 – First-aid Measures

- **General information:** Consult a physician by providing the SDS.
- **After inhalation:** Breath in fresh air. If cannot breathe, give artificial respiration and consult a physician.
- **After skin contact:** Immediately wash with soap and plenty of water and rinse thoroughly. Consult a physician.
- **After eye contact:** Rinse opened eyes with plenty of water for at least 15 minutes. Remove contact lenses, if present and easy to do so. Consult a physician.
- **After swallowing:** Not expected to present a significant ingestion hazard under anticipated conditions of normal use. If you feel unwell, seek medical advice.

Article 5 - Fire-fighting Measures

- **Suitable extinguishing media:** Use water spray, extinguishing powder, carbon dioxide, or other appropriate measure that is suitable to the environment.
- **Specific hazards arising from the substance or mixture:** None known.
- **Special protective equipment and precautions for fire-fighters:** Self-contained breathing apparatus if necessary.

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Article 6 – Accidental Release Measures

- **Personal precautions, protective equipment and emergency procedures:** Apply standard laboratory practices and personal protective equipment. Avoid breathing vapors, mist, or gas. Ensure adequate ventilation.
- **Environmental precautions:** Do not allow to enter drains.
- **Methods and materials for containment and cleaning up:** Absorb on sand or vermiculite and place in closed containers for disposal.

Article 7 - Handling and Storage

- **Precautions for safe handling:** Wear chemical safety goggles and compatible chemical-resistant gloves. Avoid inhalation, contact with eyes, skin or clothing.
- **Conditions for safe storage:** Store in a dry and well-ventilated place in -70 °C. Keep container upright and tightly closed.

Article 8 - Exposure Controls/Personal Protection

- **Components with limit monitoring values at workplace:**
NA
- **Appropriate engineering controls:**
Apply adequate ventilation including mechanical exhaust or laboratory fume hood. Follow standard laboratory practices.
- **Individual protection measures:**
Respiratory protection:
Use appropriate respirator if there is inadequate ventilation by following the government standards.
Hand protection:
Wear gloves and use proper glove removal technique to avoid skin contact. Discard gloves after use by following the applicable laboratory regulations. Wash and dry hands.
Eye/face protection:
Safety goggles with side-shields approved under appropriate government standards.
Skin/body protection:
Use appropriate clothing, footwear and any additional protection measures to protect from splashing or contamination.

Article 9 – Physical and Chemical Properties

Appearance: Colorless fluid.	Danger of explosion: Product does not present an explosion hazard.
Odour/Odour Threshold: Not determined.	Explosion limits: Not available.
pH: Not available.	Decomposition temperature: Not available.
Melting point/freezing point: Not determined.	Vapor pressure at 20 °C: Not available.
Boiling point/Boiling range: >100 °C.	Density: Not determined.
Flash point: > 100 °C.	Relative density: Not determined.
Flammability (solid, gaseous): Not determined.	Vapor density: Not determined.
Ignition temperature: Not determined.	Evaporation rate: Not determined.
Auto-igniting: Product is not self-igniting.	Solubility in / Miscibility with Water: Fully miscible.

Article 10 - Stability and Reactivity

- **Reactivity:** Stable under recommended transport and storage conditions.
- **Chemical stability:** Stable under recommended transport and storage conditions.
- **Possible hazardous reactions:** No dangerous reactions known.
- **Conditions to avoid:** Heat and moisture.
- **Incompatible materials:** Not determined.
- **Hazardous decomposition products:** Not determined.

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Article 11 - Toxicological Information

- **Acute toxicity:** Not available.
- **LD/LC50:** Not available.
- **Skin corrosion/irritation:** Not available.
- **Serious eye damage/eye irritation:** Not available.
- **Respiratory or skin sensitization:** Not available.
- **Germ cell mutagenicity:** Not available.
- **Carcinogenicity:** No components are listed in IARC, or NTP, or OSHA, or ACGIH.
- **Reproductive toxicity:** Not available.
- **Teratogenicity:** Not available.
- **Specific target organ toxicity - single exposure/ - repeated exposure (GHS):** Not available.
- **Aspiration hazard:** Not available.
- **Potential health effects:**
 - Inhalation:** No data available
 - Ingestion:** No data available
 - Skin:** No data available
 - Eyes:** No data available
- **Signs and Symptoms of Exposure:** No data available
- **Synergistic effects:** Not available.

Article 12 - Ecological Information

- **Eco-toxicity:** No data available.
- **Biodegradability:** Not applicable.
- **Bio-accumulative potential:** Not applicable.
- **Mobility in soil:** Not applicable.
- **PBT and vPvB assessment:** Not applicable.
- **Other adverse effects:** Not applicable.

Article 13 - Disposal Considerations

- **Disposal methods:** In accordance to applicable national, regional, or local laws and regulations. For additional handling information and protection of employees please refer to Article 7 and 8.
- **Contaminated packaging:** Disposal should be made in accordance to official regulations. Use water or cleansing agents to clean the area.

Article 14 - Transport Information

- **DOT:** Not dangerous goods.
- **IMDG:** Not dangerous goods.
- **IATA:** Not dangerous goods.

Article 15 - Regulatory Information

- **WHMIS Classification:** Non-hazardous.
- **GHS label elements:** Not applicable.
- **Signal word:** Not applicable.
- **Hazard statements:** Not applicable.

Article 16 - Other Information

The above information is believed to be correct but does not purport to be all-inclusive and shall be used only as a guide. SignalChem shall not be held liable for any damage resulting from handling or from contact with the above product. See the Technical Specification, Packing Slip, Invoice, and Product Catalog for additional terms and conditions of sale.

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