Product Manual

Cas9 (CRISPR Associated Protein 9) Activity Assay Kit *(S. pyogenes)*

Catalog Number

PRB-5206 **20 assays**

FOR RESEARCH USE ONLY
Not for use in diagnostic procedures

CELL BIOLABS, INC.
Creating Solutions for Life Science Research

Introduction

Cas9 (CRISPR associated protein 9) is an RNA-guided DNA endonuclease. This enzyme associates with the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) adaptive immunity system in various types of bacteria including *Streptococcus pyogenes*. Cas9 is able to unwind foreign DNA (such as plasmid DNA or invading bacteriophage DNA) and then checks for sites complementary to the 20-base pair spacer region of the guide RNA. If the DNA substrate is complementary to the guide RNA, Cas9 cuts up invading DNA.

The Cas9 protein has gained worldwide attention as a genome engineering tool to cause site-directed double strand breaks in DNA. Resulting DNA breaks can inactivate genes or introduce heterologous genes through non-homologous end joining and homologous recombination, respectively, in many laboratory model organisms. Furthermore, Cas9 can cleave nearly any sequence complementary to its associated guide RNA. Both gene deletion and gene replacement have been demonstrated using the CRISPR/Cas9 system in human cells.

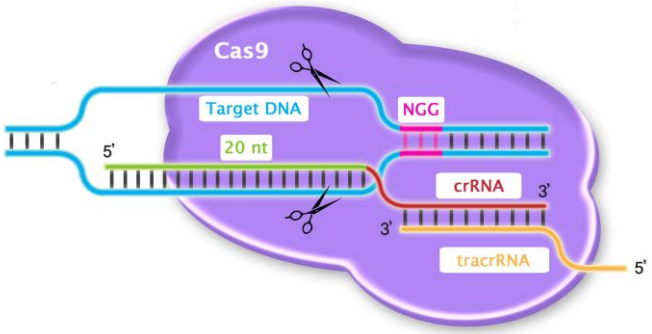

Figure 1: CRISPR/Cas9 DNA Editing.

Cell Biolabs' Cas9 Activity Assay Kit (*S. pyogenes*) is an enzymatic assay developed to test the activity of *S. pyogenes* Cas9 protein in cell lysate samples. The kit has a detection sensitivity limit as low as 10 µg/mL of active Cas9 protein. Each kit provides sufficient reagents to perform up to 20 assays including controls.

Related Products

1. PRB-5079 Cas9 (CRISPR Associated Protein 9) ELISA kit (*S. pyogenes*)
2. PRB-5204 Cas9 (CRISPR Associated Protein 9) ELISA kit (*S. aureus*)
3. PRB-5206 Cas 9 (CRISPR Associated Protein 9) Activity kit (*S. aureus*)
4. AKR-121: GFP ELISA Kit
5. AKR-122: RFP ELISA Kit

Kit Components (shipped on dry ice)

1. 10X Assay Buffer (Part No. 52031D): One 50 µL vial
2. sgRNA (Part No. 52061D): One 40 µL vial
3. Target DNA (Part No. 52062D): One 200 µL vial
4. SpCas9 Enzyme (Part No. 52063D): One 40 µL vial of 0.5 mg/mL recombinant *S. pyogenes* Cas9
5. Proteinase K (Part No. 52035D): One 40 µL vial of Proteinase K at 800 units/mL

Materials Not Supplied

1. Cell lysate
2. RNase-free water
3. Gel electrophoresis apparatus
4. Agarose
5. DNA stain – SYBR green or ethidium bromide
6. DNA cleanup kit (optional)
7. Gel Loading buffer

Storage

Upon receipt, store the kit at -80ºC. To avoid multiple freeze/thaw cycles, aliquot reagents.

Assay Precautions

- Wear gloves for all steps in the assay protocol.
- Use RNase-free water and clean equipment (preferably RNase-free) for the assay.

Preparation of Samples

The following recommendations are only guidelines and may be altered to optimize or complement the user's experimental design.

- Cell Lysates: Cells can be lysed in lysis buffer containing 20 mM HEPES pH 7.5, 100 mM KCl, 5 mM $MgCl_2$, 1 mM DTT, 5% Glycerol, 0.1% Triton X-100. Collect supernatant after centrifugation at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store lysate samples at -80ºC for up to three months.

  Note:
  1) This assay is not compatible to RIPA buffer or buffer containing SDS or deoxycholate.
  2) This assay has a sensitive limit of 10 µg/mL SpCas9. Lysates should be measured to determine concentration of SpCas9 either by ELISA (Cat No. PRB-5079) or by some other method.

Assay Protocol

1. Prepare six microcentrifuge tubes labeled 1 through 6.
2. To each tube, add the required volume of sgRNA, RNase-free water and either recombinant SpCas9 Enzyme control or cell lysate samples according to Table 1.

Tube	sgRNA	SpCas9 Enzyme Control	Cell lysate	RNase-free water	Total Volume
1	-	-	-	12.5 µL	**12.5 µL**
2	2 µL	-	-	10.5 µL	**12.5 µL**
3	-	2 µL	-	10.5 µL	**12.5 µL**
4	2 µL	2 µL	-	8.5 µL	**12.5 µL**
5	-	-	10.5 µL	2 µL	**12.5 µL**
6	2 µL	-	10.5 µL	-	**12.5 µL**

Table 1. Preparation of Enzyme Control and Cell Lysate Samples.

3. Incubate tubes at room temperature for 10 minutes.
4. Add 2.5 µL of 10X Assay Buffer and 10 µL Target DNA to each tube and mix thoroughly.
5. Incubate tubes at 37ºC for 1 hour.
6. Add 2 µL Proteinase K to each tube. Mix and centrifuge contents to the bottom of the tube.
7. Incubate tubes at 37ºC for 15 minutes.

   Optional: For cell lysate samples, after Proteinase K treatment, DNA fragments should be purified using a DNA clean up kit. This step is useful to remove background exposure after gel electrophoresis and staining.
8. Add gel loading buffer (not provided) and load entire volume onto a 1.5% agarose-TBE gel. Run the gel at 80-150 V until the dye line is approximately 75-80% of the way down the gel.
9. Stain gel with SYBR green or ethidium bromide (not provided) and visualize gel under blue light or UV light.

   Note: Expected DNA sizes before and after Cas9 digestion are as followed: 368 bp (uncut Target DNA); 184 bp each (digested Cas9 products)

Example of Results

The following figures demonstrate typical results with the SpCas9 Activity Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

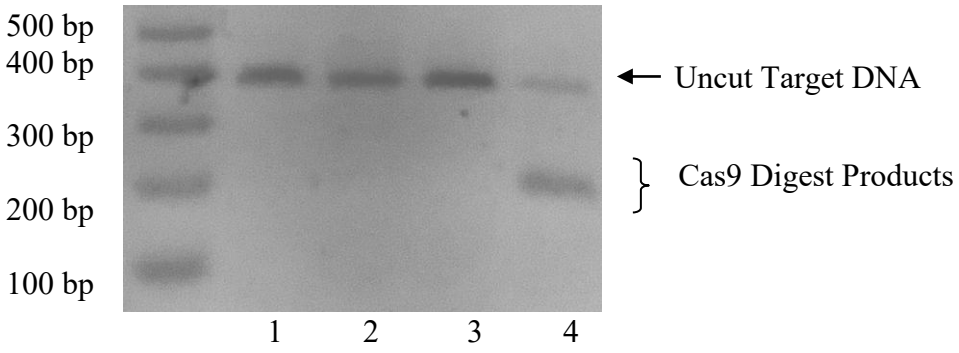

Figure 2: Cas9 Activity Assay. Target DNA only (Lane 1) was incubated with *S. pyogenes* Cas 9 (SpCas9) only (Lane 2), single guide RNA (sgRNA) only (Lane 3), or SpCas9 and sgRNA (Lane 4).

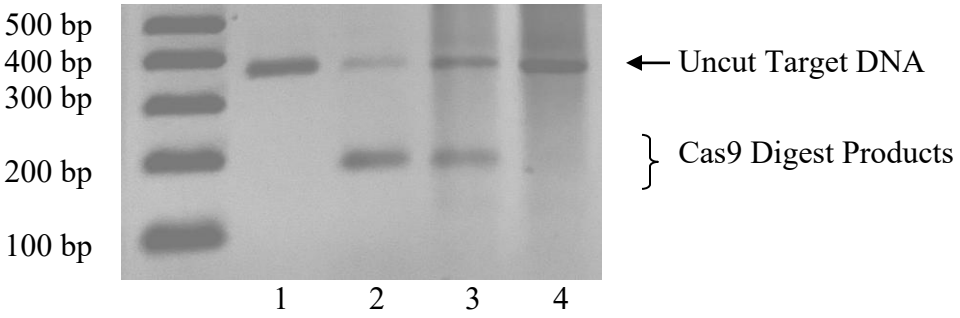

Figure 3: Detection of Cas9 activity in transfected lysate. 293T cells were transfected with a plasmid expressing SpCas9 or an empty vector plasmid. After 24-48 hours, cells were lysed in lysis buffer (as mentioned above). Target DNA (Lane 1) in the presence of sgRNA was incubated with recombinant purified SpCas9 (Lane 2), 100 µg of transfected 293T lysate containing SpCas9 (Lane 3) or 100 µg of transfected 293T lysate containing an empty vector (Lane 4). After incubation in Proteinase K, DNA fragments were cleaned and concentrated in a DNA Spin column prior to loading onto an 1.5% agarose gel.

References

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Warranty

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