Product Manual

Cas9 (CRISPR Associated Protein 9) ELISA Kit (*S. aureus*)

Catalog Number

PRB-5204 **96 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 aureus*. 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.

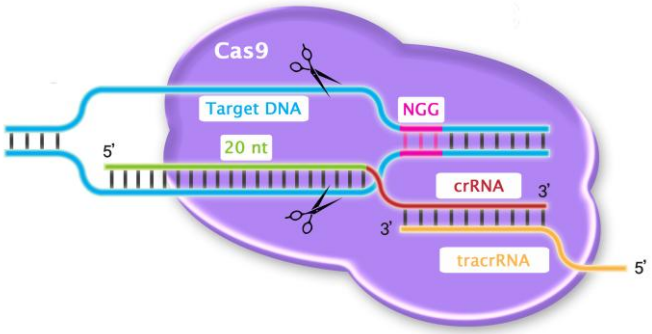

Figure 1: CRISPR/Cas9 DNA Editing.

Cell Biolabs' Cas9 ELISA Kit (S. aureus) is an enzyme immunoassay developed for the detection and quantitation of *S. aureus* Cas9 (SaCas9) in cell or tissue lysate samples. The kit has a detection sensitivity limit of 3.9 ng/mL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

1. PRB-5079 Cas9 ELISA kit
2. AKR-120: GFP Quantitation Kit, Fluorometric
3. AKR-121: GFP ELISA Kit
4. AKR-122: RFP ELISA Kit
5. AKR-130: His-Tag Protein ELISA Kit

Kit Components

Box 1 (shipped at room temperature)

1. 96-well Protein Binding Plate (Part No. 231001): One 96-well strip plate.
2. 100X Anti-SaCas9 Coating Antibody (Part No. 52044D): One 100 µl vial.
3. Biotinylated Anti-SaCas9 Antibody (1000X) (Part No. 52042D): One 10 µL vial.
4. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
5. Assay Diluent (Part No. 310804): One 50 mL bottle.
6. 5X SaCas9 Sample Buffer Diluent (Part No. 52045B): One 20 ml bottle.
7. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
8. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
9. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. SaCas9 Standard (Part No. 52043D): One 25 µL vial of 25 µg/mL recombinant *S. aureus* Cas9.

Materials Not Supplied

1. Cell or tissue lysate
2. PBS containing 0.1% BSA
3. RIPA buffer

Storage

Upon receipt, aliquot and store the SaCas9 Standard at -80ºC to avoid multiple freeze/thaw cycles. Store the Biotinylated Anti-SaCas9 Antibody and 100X Anti-SaCas9 coating antibody at -20ºC. Store all other components at 4ºC.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- 1X SaCas9 Sample Buffer Diluent: Dilute the 5X SaCas9 Sample Buffer Diluent to 1X with deionized water. Stir to homogeneity.
- Biotinylated Anti-SaCas9 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-SaCas9 antibody and Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.
- Anti-SaCas9 Antibody Coated Plate:

  Note: Anti-SaCas9 coated wells are not stable and should be used within 24 hr after coating. Only coat the number of wells to be used immediately. It is recommended that the user prepare the standards and samples during the blocking of the Anti-SaCas9 Antibody coated plate.

  1. Immediately before use, dilute the 100X Anti-SaCas9 Coating Antibody 1:100 in 1X PBS. Example: Add 20 µL to 1980 µl LX PBS. Vortex thoroughly.

2. Add 100 µL of the mixture to each well to be tested and incubate overnight at 4ºC. Remove the Anti-SaCas9 Antibody coating solution and blot the plate on paper towels to remove excess fluid.
3. Add 200 µl of Assay Diluent to each well to be tested and block for 2 hr at room temperature. Remove the solution and wash the wells three times with 200 µl 1X PBS and blot the plate on paper towels to remove excess fluid. Use the plate immediately after the last PBS wash.

Preparation of Standard Curve

Prepare a dilution series of SaCas9 standards in the concentration range of 0 to 250 ng/mL into 1X SaCas9 Sample Buffer Diluent (Table 1).

Standard Tubes	25 µg/mL SaCas9 Standard (µL)	1X SaCas9 Sample Buffer Diluent (µL)	SaCas9 (ng/mL)
1	5	495	250
2	250 of Tube #1	250	125
3	250 of Tube #2	250	62.5
4	250 of Tube #3	250	31.3
5	250 of Tube #4	250	15.6
6	250 of Tube #5	250	7.8
7	250 of Tube #6	250	3.9
8	0	250	0

Table 1. Preparation of *S. aureus* Cas9 Standards

Preparation of Samples

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

- Cell or Tissue Lysate: Sonicate or homogenize sample in Lysis Buffer such as RIPA buffer (25 mM Tris•HCl pH 7.6, 150 mM NaCl, 1% NP-40, 1% sodium deoxycholate, 0.1% SDS) and centrifuge at 10,000 x g for 10 minutes at 4ºC. Assay immediately or store samples at -80ºC for up to three months. Dilute samples in SaCas9 Sample Buffer Diluent as needed.

Assay Protocol

1. Add 100 µL of SaCas9 unknown sample or standard to the SaCas9 Antibody Coated Plate. Each SaCas9 unknown sample, standard and blank should be assayed in duplicate.
2. Incubate for 2 hours in a 37ºC incubator.
3. Wash strip wells 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, empty wells and tap microwell strips on absorbent pad or paper towel to remove excess 1X Wash Buffer.
4. Add 100 µL of the diluted Biotinylated Anti-SaCas9 Antibody to each well. Incubate at room temperature for 1 hour on an orbital shaker.
5. Wash the strip wells 3 times according to step 3 above.

6. Add 100 µL of the diluted Streptavidin-Enzyme Conjugate to each well. Incubate at room temperature for 1 hour on an orbital shaker.
7. Wash the strip wells 3 times according to step 3 above. Proceed immediately to the next step.
8. Warm Substrate Solution to room temperature. Add 100 µL of Substrate Solution to each well, including the blank wells. Incubate at room temperature for 2-30 minutes on an orbital shaker.

   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance of each microwell on a spectrophotometer using 450 nm as the primary wave length.

Example of Results

The following figures demonstrate typical results with the Cas9 ELISA Kit (*S. aureus*). One should use the data below for reference only. This data should not be used to interpret actual results.

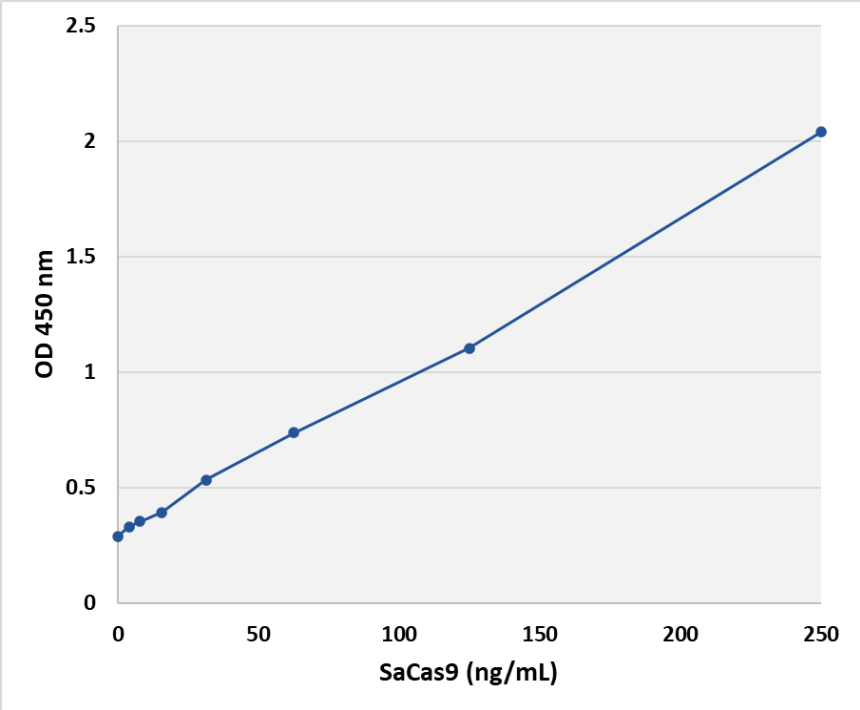

Figure 2: SaCas9 ELISA Standard Curve.

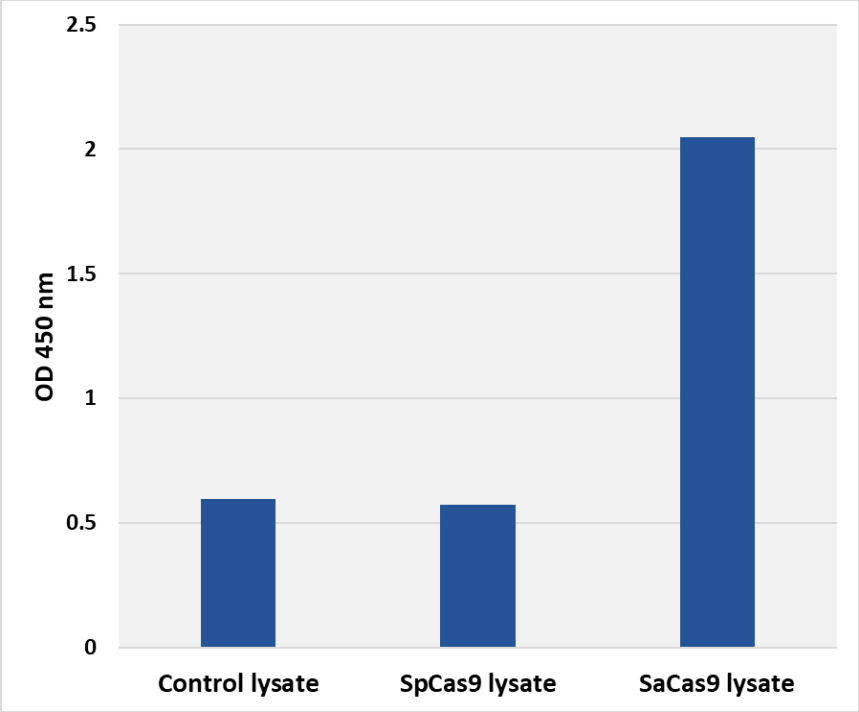

Figure 3: Detection of SaCas9 in Transfected 293 cells. Cells were transiently transfected with an empty vector (control) or a Cas9 mammalian expression vector encoding *S. pyogenes* Cas9 or *S. aureus* Cas9. After 48 hours, cells were lysed and protein concentration was determined. The Cas9 ELISA kit (*S. aureus*) was performed with protein lysate from control (control lysate), *S. pyogenes* Cas9 (SpCas9 lysate), or *S. aureus* Cas9 (SaCas9 lysate).

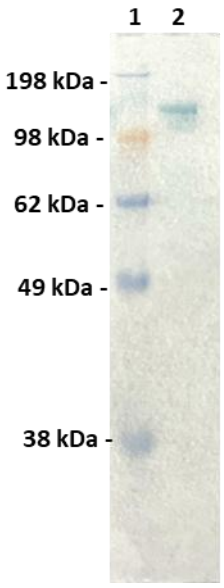

Figure 4: Purification of Recombinant SaCas9 protein. Lane 1: MW standard; Lane 2: Purified recombinant SaCas9 used as immunogen to produce the ELISA antibodies.

References

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