Product Manual

Cas9 (CRISPR Associated Protein 9) ELISA Kit

Catalog Number

PRB-5079 **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 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.

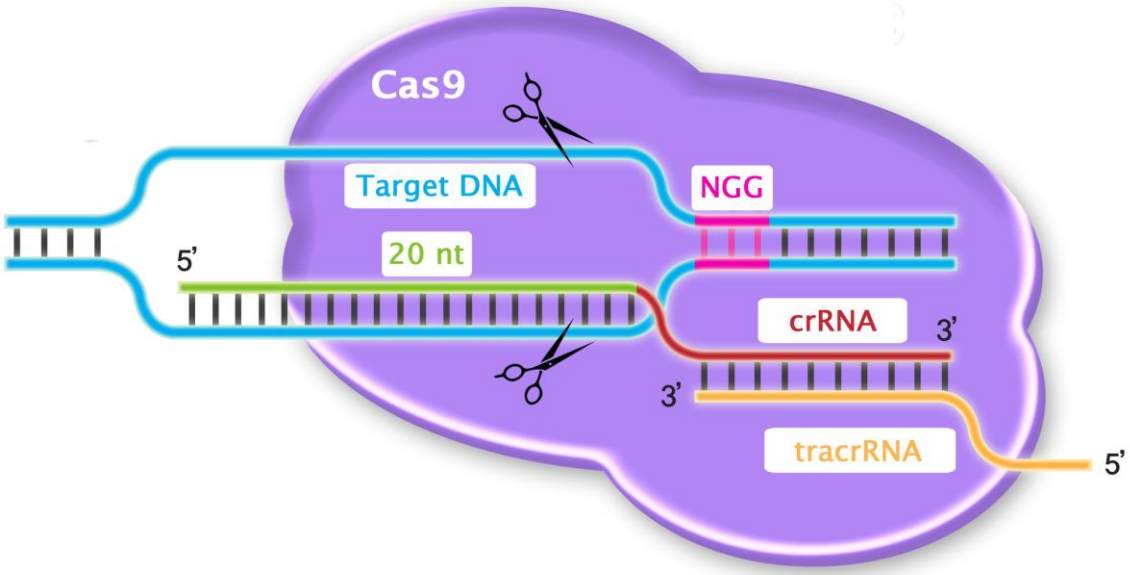

Figure 1: CRISPR/Cas9 DNA Editing.

Cell Biolabs' Cas9 ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of *S. pyogenes* Cas9 in cell or tissue lysate samples. The kit has a detection sensitivity limit of 1.5 ng/mL Cas9. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Related Products

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

Kit Components

Box 1 (shipped at room temperature)

1. Anti-Cas9 Antibody Coated Plate (Part No. 50791B): One 96-well strip plate (8 x 12).
2. Biotinylated Anti-Cas9 Antibody (1000X) (Part No. 50792C): One 10 µL vial.
3. Streptavidin-Enzyme Conjugate (Part No. 310803): One 20 µL vial.
4. Assay Diluent (Part No. 310804): One 50 mL bottle.
5. 10X Wash Buffer (Part No. 310806): One 100 mL bottle.
6. Substrate Solution (Part No. 310807): One 12 mL amber bottle.
7. Stop Solution (Part. No. 310808): One 12 mL bottle.

Box 2 (shipped on blue ice packs)

1. Cas9 Standard (Part No. 50793D): One 50 µL vial of 5 µg/mL recombinant *S. pyogenes* Cas9.

Materials Not Supplied

1. Cell or tissue lysate
2. PBS containing 0.1% BSA
3. 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips
4. 50 µL to 300 µL adjustable multichannel micropipette with disposable tips
5. Multichannel micropipette reservoir
6. Microplate reader capable of reading at 450 nm (620 nm as optional reference wave length)
7. RIPA buffer

Storage

Upon receipt, aliquot and store the Cas9 Standard at -80ºC to avoid multiple freeze/thaw cycles. Store the Biotinylated Anti-Cas9 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.
- Biotinylated Anti-Cas9 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use dilute the Biotinylated Anti-Cas9 antibody and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

Prepare a dilution series of Cas9 standards in the concentration range of 0 to 100 ng/mL into Assay Diluent (Table 1).

Standard Tubes	5 µg/mL Cas9 Standard (µL)	Assay Diluent (µL)	Cas9 (ng/mL)
1	10	490	100
2	250 of Tube #1	250	50
3	250 of Tube #2	250	25
4	250 of Tube #3	250	12.5
5	250 of Tube #4	250	6.25
6	250 of Tube #5	250	3.13
7	250 of Tube #6	250	1.56
8	0	250	0

Table 1. Preparation of *S. Pyogenes* 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 PBS containing 0.1% BSA as needed.

Assay Protocol

1. Add 100 µL of Cas9 unknown sample or standard to the Anti-Cas9 Antibody Coated Plate. Each Cas9 unknown sample, standard and blank should be assayed in duplicate.
2. Incubate at room temperature for 1 hour on an orbital shaker.
3. Wash microwell strips 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-Cas9 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 on an orbital shaker. Actual incubation time may vary from 2-30 minutes.

   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. One should use the data below for reference only. This data should not be used to interpret actual results.

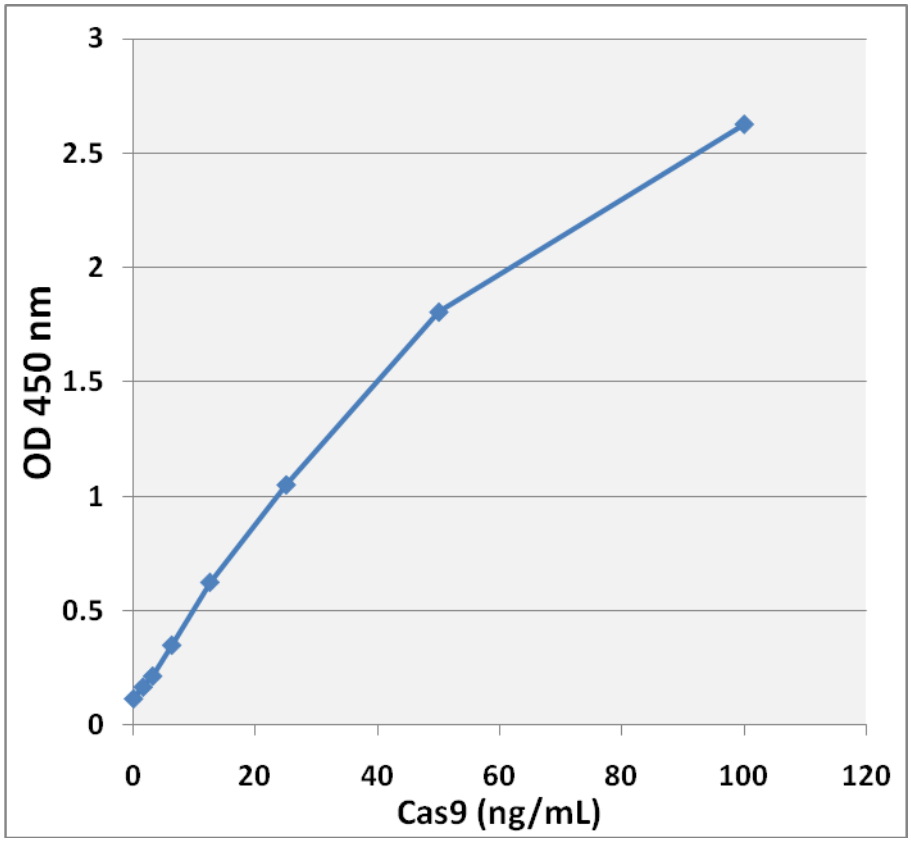

Figure 2: Cas9 ELISA Standard Curve.

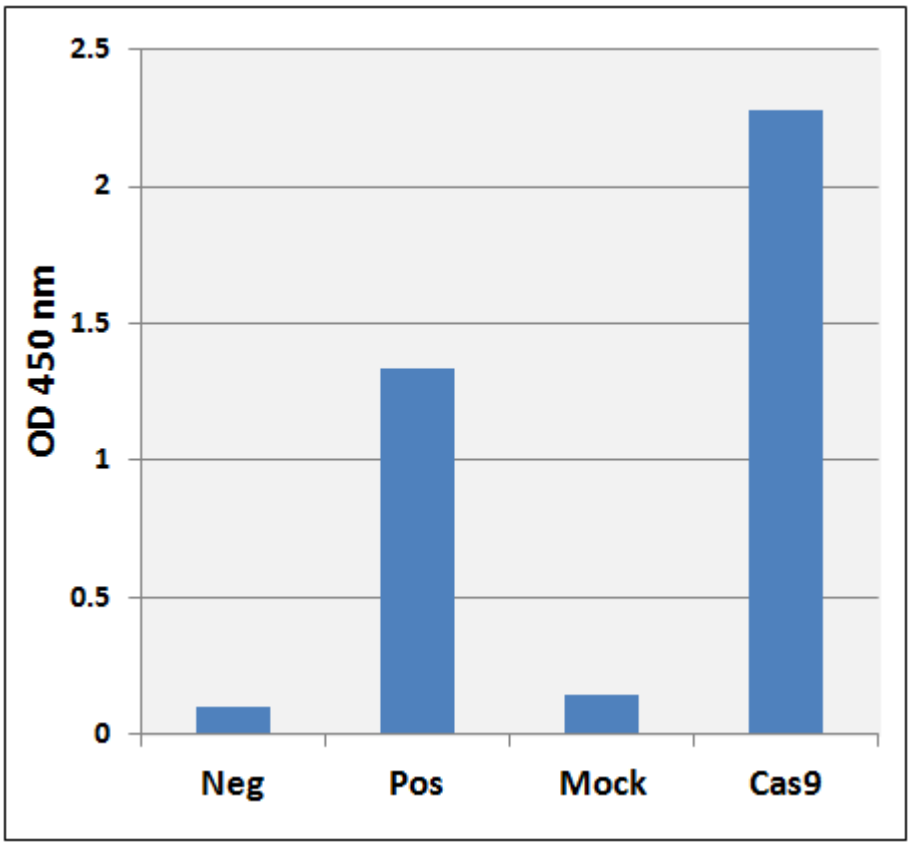

Figure 3: Detection of Cas9 in Transfected 293 cells. Cells were transiently transfected with a Cas9 mammalian expression vector or mock transfected. After 48 hours, cells were lysed in RIPA buffer and protein concentration was determined. The Cas9 ELISA kit was performed in the absence of cell lysate (Neg), the presence of 50 ng/mL Cas9 Nuclease (Pos) (NEB catalog number M0386), the presence of 10 µg protein lysate of mock transfected (Mock), or Cas9 transfected (Cas9).

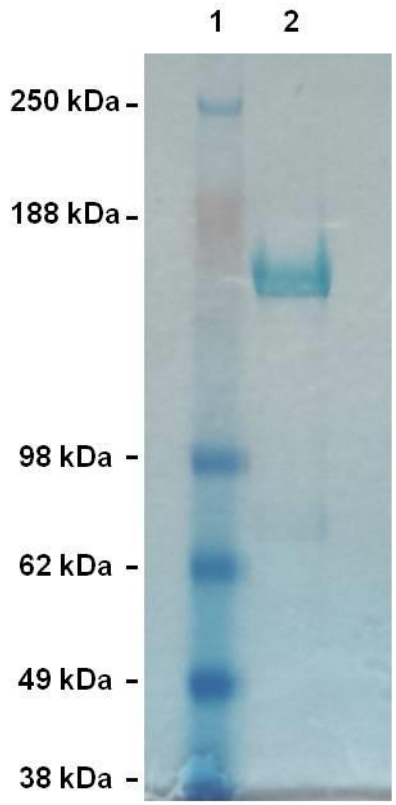

Figure 4: Purification of Recombinant Cas9 protein. Lane 1: SeeBlue Plus2 MW standard (Invitrogen); Lane 2: Ni-NTA Elution Fraction for Recombinant Cas9. Purified recombinant Cas9 protein was used as immunogen to produce the ELISA antibodies.

Appendix

Recombinant Cas9 Sequence: Full Length Cas9 is underlined.

MGSSHHHHHHSSGLVPRGSHMASMTGGQQMGRGSEFELRRQACGRMDKKYSIGLDIGTNSVGWAVITD
EYKVPSKKFKVLGNTDRHSIKKNLIGALLFDSGETAEATRLKRTARRRYTRRKNRICYLQEIFSNEMA
KVDDSFFHRLEESFLVEEDKKHERHPIFGNIVDEVAYHEKYPTIYHLRKKLVDSTDKADLRLIYLALA
HMIKFRGHFLIEGDLNPDNSDVDKLFIQLVQTYNQLFEENPINASGVDAKAILSARLSKSRRLENLIA
QLPGEKKNGLFGNLIALSLGLTPNFKSNFDLAEDAKLQLSKDTYDDDLDNLLAQIGDQYADLFLAAKN
LSDAILLSDILRVNTEITKAPLSASMIKRYDEHHQDLTLLKALVRQQLPEKYKEIFFDQSKNGYAGYI
DGGASQEEFYKFIKPILEKMDGTEELLVKLNREDLLRKQRTFDNGSIPHQIHLGELHAILRRQEDFYP
FLKDNREKIEKILTFRIPYYVGPLARGNSRFAWMTRKSEETITPWNFEEVVDKGASAQSFIERMTNFD
KNLPNEKVLPKHSLLYEYFTVYNELTKVKYVTEGMRKPAFLSGEQKKAIVDLLFKTNRKVTVKQLKED
YFKKIECFDSVEISGVEDRFNASLGTYHDLLKIIKDKDFLDNEENEDILEDIVLTLTLFEDREMIEER
LKTYAHLFDDKVMKQLKRRRYTGWGRLSRKLINGIRDKQSGKTILDFLKSDGFANRNFMQLIHDDSLT
FKEDIQKAQVSGQGDSLHEHIANLAGSPAIKKGILQTVKVVDELVKVMGRHKPENIVIEMARENQTTQ
KGQKNSRERMKRIEEGIKELGSQILKEHPVENTQLQNEKLYLYYLQNGRDMYVDQELDINRLSDYDVD
HIVPQSFLKDDSIDNKVLTRSDKNRGKSDNVPSEEVVKKMKNYWRQLLNAKLITQRKFDNLTKAERGG
LSELDKAGFIKRQLVETRQITKHVAQILDSRMNTKYDENDKLIREVKVITLKSKLVSDFRKDFQFYKV

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REINNYHHAHDAYLNAVVGTALIKKYPKLESEFVYGDYKVYDVRKMIAKSEQEIGKATAKYFFYSNIM
NFFKTEITLANGEIRKRPLIETNGETGEIVWDKGRDFATVRKVLSMPQVNIVKKTEVQTGGFSKESIL
PKRNSDKLIARKKDWDPKKYGGFDSPTVAYSVLVVAKVEKGKSKKLKSVKELLGITIMERSSFEKNPI
DFLEAKGYKEVKKDLIIKLPKYSLFELENGRKRMLASAGELQKGNELALPSKYVNFLYLASHYEKLKG
SPEDNEQKQLFVEQHKHYLDEIIEQISEFSKRVILADANLDKVLSAYNKHRDKPIREQAENIIHLFTL
TNLGAPAAFKYFDTTIDRKRYTSTKEVLDATLIHQSITGLYETRIDLSQLGGDGGSGPPK
KKRKVYPYDVPDYAC

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Recent Product Citations

1. Mills, C. et al. (2022). A Novel CRISPR Interference Effector Enabling Functional Gene Characterization with Synthetic Guide RNAs. *CRISPR J*. doi: 10.1089/crispr.2022.0056.
2. Van Cleemput, J. et al. (2021). CRISPR/Cas9-Constructed Pseudorabies Virus Mutants Reveal the Importance of UL13 in Alphaherpesvirus Escape from Genome Silencing. *J Virol*. **95**(6):e02286-20. doi: 10.1128/JVI.02286-20.
3. Lee, M.H. et al. (2021). Cellular reprogramming with multigene activation by the delivery of CRISPR/dCas9 ribonucleoproteins via magnetic peptide-imprinted chitosan nanoparticles. *Mater Today Bio*. **9**:100091. doi: 10.1016/j.mtbio.2020.100091.

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