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CRISPR/Cas9 protein ELISA Kit

Catalog number: NB-E1372HS (96 wells)

The kit is designed to quantitatively detect

the level of S. pyogenes Cas9 in cell or tissue lysate samples.

Manufactured and Distributed by:

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Important notes

Before using this product, please read this manual carefully; after reading the subsequent contents of this manual, please note the following specially :

- The operation should be carried out in strict accordance with the provided instructions.
- Store the unused strips in a sealed foil bag at 2-8°C.
- Always avoid foaming when mixing or reconstituting protein solutions.
- Pipette reagents and samples into the center of each well, avoid bubbles.
- The samples should be transferred into the assay wells within 15 minutes of dilution.
- We recommend that all standards, testing samples are tested in duplicate.
- Using serial diluted sample is recommended for first test to get the best dilution factor.
- If the blue color develops too light after 15 minutes incubation with the substrates, it may be appropriate to extend the incubation time. (Do not over-develop)
- Avoid cross-contamination by changing tips, using separate reservoirs for each reagent.
- Avoid using the suction head without extensive wash.
- Do not mix the reagents from different batches.
- Stop Solution should be added in the same order of the Substrate Solution.
- TMB developing agent is light-sensitive. Avoid prolonged exposure to the light.

Intended use

The 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 31 pg/mL Cas9. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples .

Standard range	**78 - 5000 pg/ml**
Sensitivity	**31 pg/ml**
Assay time	**3.0 hours**
Validity	**Six months**
Store at	**2-8 °C**

Assay principle

This CRISPR/Cas9 protein enzyme linked immunosorbent assay applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with a monoclonal antibody specific for CRISPR/Cas9 protein. Standards or samples are then added to the microtiter plate wells and CRISPR/Cas9 protein if presents, will bind to the antibody pre-coated wells. In order to quantitatively determine the amount of CRISPR/Cas9 protein present in the sample, a standardized preparation of biotin-conjugated antibody, specific for CRISPR/Cas9 protein are added to each well to "sandwich" the CRISPR/Cas9 protein immobilized on the plate. The microtiter plate undergoes incubation, and then the wells are thoroughly washed to remove all unbound components. Streptavidin-HRP was added and incubated, washed. Next, substrate solution is added to each well. The enzyme (HRP) and substrate are allowed to react over a short incubation period. Only those wells that contain CRISPR/Cas9 protein and enzyme-conjugated antibody will exhibit a change in colour. The enzyme-substrate reaction is terminated by addition of a sulphuric acid solution and the colour change is measured spectrophotometrically at a wavelength of 450 nm.

Materials supplied

S/N	Item/Name	Size	shipped	Storage
1	Anti-Cas9 Antibody Coated Plate	96 well	RT	4°C
2	Cas9 Standard (Lyophilized powder)	25 ng	Blue Ice Packs	-80°C
3	Biotinylated Anti-Cas9 Antibody (1000X)	10 µL	Blue Ice Packs	-20°C
4	Streptavidin-Enzyme Conjugate (1000X)	20 µL	Blue IcePacks	4°C
5	Assay Diluent	50 ml	RT	4°C
6	Substrate Solution	12 ml	RT	4°C
7	Stop Solution	12 ml	RT	4°C
8	10 X Wash Solution	100 ml	RT	4°C
9	Closure plate membrane	2	RT	N/A
10	Package insert	1	RT	N/A

Note: After dilution,the standard (S1-S6) concentration was followed by: 78, 156, 312, 625, 1250, 2500, and 5000 pg/ml

Materials required but not supplied

- Cell or tissue lysate.
- Standard plate reader capable of measuring absorbance at 450 nm (620 nm as optional reference wave length).
- PBS containing 0.1% BSA.
- 10 µL to 1000 µL adjustable single channel micropipettes with disposable tips .
- 50 µL to 300 µL adjustable multichannel micropipette with disposable tips.
- Multichannel micropipette reservoir.
- RIPA buffer.

Storage

Upon receipt, aliquot and store the Cas9 Standard at -80 ^{o} C to avoid multiple freeze the Biotinylated Anti-Cas9 Antibody at -20^{o} C. Store all other components at 4^{o} C.

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) using sonication & vortex procedures ,and centrifuge at 10,000 x g for 10 minutes at 4^{o} C to clear the lysate. Assay immediately or store samples at -80^{o} C for up to three months. Dilute samples in PBS containing 0.1% BSA as needed.

Preparation of Reagents

- 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity.
- For the following three components: Cas9 Standard, Biotinylated Anti-Cas9 Antibody and Streptavidin-Enzyme Conjugate in this kit, centrifuge with a refrigerated centrifuge for 2 minutes before opening the lid.
- Biotinylated Anti-Cas9 Antibody and Streptavidin-Enzyme Conjugate: Immediately before use briefly spin to recover all the liquid , dilute the Biotinylated Anti -Cas 9 antibody and the Streptavidin-Enzyme Conjugate 1:1000 with Assay Diluent. Do not store diluted solutions.

Preparation of Standard Curve

For Cas9 protein,please add 50µl of nuclease-free water to reconstitute for 10 min. Mix well by pipetting . Then prepare a dilution series of Cas9 standards in the concentration range of 0 to 5000 pg/ mL into Assay Diluent (Table 1).

Standard Tubes	Cas9 Standard (µL)	Assay Diluent (µL)	Cas9 (pg/mL)
1	5 (500 ng/ml)	495	5000
2	250 of Tube #1	250	2500
3	250 of Tube #2	250	1250
4	250 of Tube #3	250	625
5	250 of Tube #4	250	312
6	250 of Tube #5	250	156
7	250 of Tube #6	250	78
8	0	250	0

Table 1. Preparation of S. Pyogenes Cas9 Standards.

Assay procedures

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 (200-500 rpm).
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 (200 -500 rpm).
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 (200-500 rpm).
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.

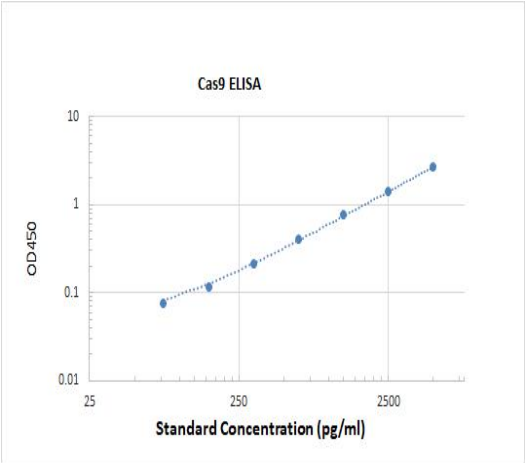
   Note: Watch plate carefully; if color changes rapidly, the reaction may need to be stopped sooner to prevent saturation.
9. Stop the enzyme reaction by adding 100 µL of Stop Solution into each well, including the blank wells. Results should be read immediately (color will fade over time).
10. Read absorbance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

Result calculation

- This standard curve is used to determine the amount of an unknown sample. Construct a standard curve by plotting the average O.D. (450 nm) for each standard on the vertical (Y) axis against the concentration on the horizontal (X) axis, and draw a best fit curve through the points on the graph.
- First, calculate the mean O.D. value for each standard and sample. All O.D. values, are subtracted by the mean value of the blank control before result interpretation. Construct the standard curve using graph paper or statistical software.
- To determine the amount in each sample, first locate the O.D. value on the Y-axis and extend a horizontal line to the standard curve. At the point of intersection, draw a vertical line to the X-axis and read the corresponding concentration.
- Any variation in operator, pipetting and washing technique, incubation time or temperature, and kit age can cause variation in result. Each user should obtain their own standard curve.

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.



Calculations of the results

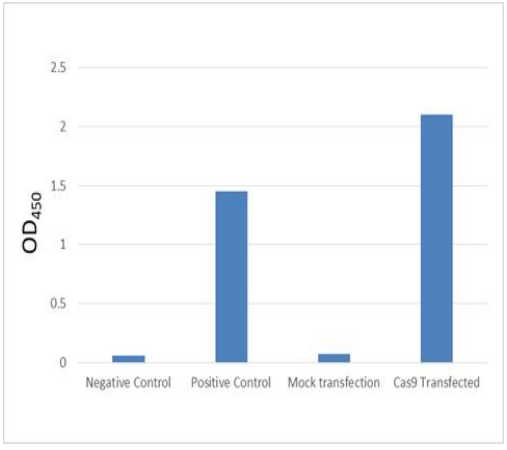
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human Cas9 concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

Figure1: Cas9 ELISA Standard Curve.

Figure 2: 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 Cas 9 ELISA kit was performed in the absence of cell lysate (Neg), the presence of 4 ng/mL Cas 9 Nuclease (Pos) (Novateinbio catalog number PR-137211), the presence of 10 µg protein lysate of mock transfected (Mock), or Cas9 transfected (Cas9).



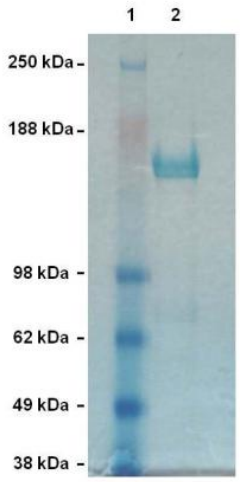


Figure2: Detection of Cas9 in Transfected 293 cells

Figure 3: Purification of Recombinant Cas9 protein.

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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.

Background

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 eterologous 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.

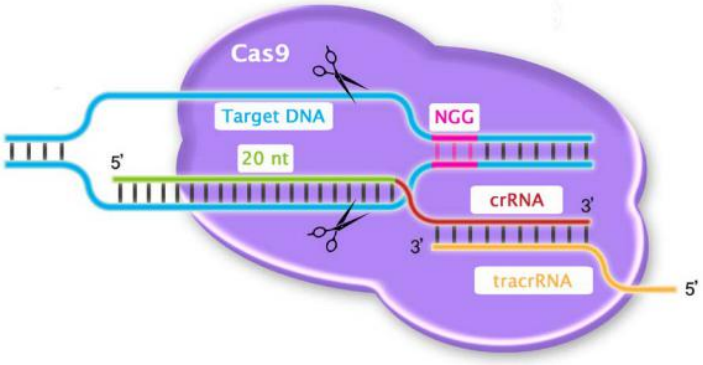

Figure 4: CRISPR/Cas9 DNA Editing.

Related Products

Product Name	Catalog Number	CRISPR-Cas9 variants
NLS-Cas9-NLS	PR-137211	Streptococcus pyogenes Cas9 wild type
NLS-Cas9-EGFP	PR-137211-E	Streptococcus pyogenes Cas9 with C-terminal EGFP
NLS-Cas9(D10A)-2NLS nickase	PR-137212B	Streptococcus pyogenes Cas9 nickas
dCas9 (D10A & H840A)	PR-137213	No nuclease activity while keeping target binding activity
NLS-dCas9-NLS	PR-137213B	No nuclease activity while keeping target binding activity
Biotinylated NLS-dCas9-NLS	PR-137213-bio	No nuclease activity while keeping target binding activity, biotinylated

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