NOVATEINBIO
Accelerates Research and Development

CRISPR/Cas9 Host Autoantibody ELISA kit

Catalog number: NB-E1372Ab (96 wells)

The kit is designed to quantitatively detect

the level of Host Autoantibodies to Cas9 Protein antigen in serum.

Manufactured and Distributed by:

Novatein Biosciences
310 W Cummings Park, Woburn, MA, 01801, USA
Phone: (617) 238-1396, Fax: (617) 380-0053
Email: Info@novateinbio.com

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 antibodies to Cas9 protein antigen in serum samples. The kit has a detection sensitivity limit of 0.2 U/mL. Each kit provides sufficient reagents to perform up to 96 assays including standard curve and unknown samples.

Standard range	**0.24 - 1000 U/ml**
Sensitivity	**0.2 U/ml**
Assay time	**100 mins**
Validity	**Six months**
Store at	**2-8 °C**

Assay principle

This anti-Cas9 protein test is an Enzyme-Linked Immunosorbent Assay to detect antibodies to Cas9 protein antigen. Purified Cas9 protein antigen is attached to a solid phase microassay well. Enzyme-Linked Immunosorbent Assays(ELISA) rely on the ability of biological materials (i.e. antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a certain host serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen- antibody complexes. Excess antibody is removed by washing.This is followed by the addition of 2nd antibody conjugated with horseradish peroxidase which then binds to the antibody- antigen complexes. The excess conjugate is removed by washing, followed by the addition of Chromogen/Substrate, tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the serum,a blue color develops. When the enzymatic reaction is stopped with 1N H_2SO4, the contents of the wells turn yellow.The color,which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.

Materials supplied

S/N	Item/Name	Size	Qty
1	96-well plate: Pre-coated with purified Cas9 protein antigen	96 wells	1
2	Serum Diluent: Contains proclin (0.1%) as a preservative.	12 ml	2
3	Antibody Diluent: Contains proclin (0.1%) as a preservative.	12 ml	1
4	Anti-Cas9 Protein Standard (1000 Unit/ml) :	1.0 ml	1
5	Horseradish-peroxidase (HRP) Conjugate(100X):	200 µl	1
6	Chromogen Solution I: H_2O_2	6.0 ml	1
7	Chromogen Solution II: Tetramethylbenzidine (TMB)	6.0 ml	1
8	Stop Solution	6.0 ml	1
9	20 X Wash Solution	25 ml	1
10	Closure plate membrane	N/A	2
11	Package insert	N/A	1

Note: After dilution,the standard (S1-S6) concentration was followed by: 0.24, 0.98, 3.9, 15.6, 62.5, 250, 1000 Units/ml

Materials required but not supplied

1. Wash bottle, automated or semi-automated microwell plate washing system.
2. Micropipettes, including multichannel, capable of accurately delivering 10-200 µL volumes. (less than 3% CV).
3. One liter graduated cylinder.
4. Paper towels.
5. Test tube for serum dilution.
6. Reagent reservoirs for multichannel pipettes.
7. Pipette tips.
8. Distilled or deionized water (dH20).
9. Timer capable of measuring to an accuracy of +/- 1 second (0 - 60 minutes).
10. Disposal basins and 0.5% sodium hypochlorite (50 mL bleach in 950 mL dH20).
11. Single or dual wavelength microplate reader with 450 nm filter. If dual wavelength is used, set the reference filter to 600-650 nm. Read the Operator's Manual or contact the instrument manufacturer to establish linearity performance specifications of the reader.

Storage

1. Store all kit component between 2°C and 8°C. The test kit may be used throughout the expiration date of the kit. Refer to the package label for the expiration date.
2. Unopened microassay plates must be stored between 2°C and 8°C. Unused strips must be immediately resealed in a sealable bag with desiccant, and returned to storage at 2°C and 8°C. If the bag is resealed with tape, the wells are stable for 30 days. If the bag is resealed with a heat sealer, the wells are stable until their labeled expiration date.

SPECIMEN COLLECTION AND PREPARATION

1. Handle all blood and serum as if capable of transmitting infectious agents.
2. Optimal performance of the ELISA kit depends upon the use of fresh serum samples (clear, non-hemolyzed, nonlipemic, non-icteric). A minimum volume of 50 µL is recommended, in case repeat testing is required. Specimens should be collected aseptically by venipuncture. Early separation from the clot prevents hemolysis of serum.
3. Store serum between 2 and 8 ^{o}C if testing will take place within five days. If specimens are to be kept for longer periods, store at -20 to -70 ^{o}C in a non-defrosting freezer. Do not use a frost free freezer because it may allow the specimens to go through freeze-thaw cycles and degrade antibody. Samples that are improperly stored or are subjected to multiple freeze-thaw cycles may yield erroneous results.
4. Serum containing visible particulate matter can be spun down utilizing slow speed centrifugation.
5. Do not use heat inactivate serum.
6. The NCCLS provides recommendations for storing blood specimens (Approved Standard - Procedures for the Handling and Processing of Blood Specimens, H18-A. 1990).12

Preparation of Reagents

- All reagents must be removed from refrigeration and allowed to come to room temperature (21 - 25 ^{o}C) before use. Return all reagents to refrigerator promptly after use.
- All samples and controls should be vortexed before use.
- Dilute 25 mL of the 20X Wash Buffer to 0.5 L with distilled and/or deionized H_20. Mix well.
- Horseradish-peroxidase (HRP) Conjugate: 110µl of Conjugate mixed with 10.89 ml Antibody Diluent before use.
- Mix chromogen solution I and chromogen solution II by the same volume before use.

Preparation of Standard Curve

For Anti-Cas9 Protein Standard , prepare a dilution series of Cas9 standards in the concentration range of 0 to 1000 Units/mL into Assay Diluent(Table1).

Standard Tubes	Anti-Cas9 Protein Standard (µL)	Serum Diluent (µL)	Cas9 (Units/mL)
1	1000 (1000 Units/ml)	0	1000
2	75 of Tube #1	225	250
3	75 of Tube #2	225	62.5
4	75 of Tube #3	225	15.6
5	75 of Tube #4	225	3.9
6	75 of Tube #5	225	0.98
7	75 of Tube #6	225	0.24
8	0	225	0

Table 1. Preparation of Anti-Cas9 Protein Standards.

Assay procedures

1. Place the desired number of strips into a microwell frame. Allow 7 standard determinations in duplicate per run. A reagent blank (RB) should be run on each assay. Check software and reader requirements for the correct Control configuration. Return unused strips to the sealable bag with desiccant, seal and immediately refrigerate.

   Plate Configuration:

Standard Description	Standard Description	Sample Description	Sample Description
STD-1000	STD-1000	#1	#1
STD-250	STD-250	#2	#2
STD-62.5	STD-62.5	#3	#3
STD-15.6	STD-15.6	#4	#4
STD-3.9	STD-3.9	#5	#5
STD-0.98	STD-0.98	#6	#6
STD-0.24	STD-0.24	#7	#7
Blank	Blank	#8	#8

2. Dilute test sera, 1:40 (e.g., 5.5 µL + 214.5 µL) in Serum Diluent. Mix well. (For manual dilutions it is suggested to dispense the Serum Diluent into the test tube first and then add the serum.)
3. To individual wells, add 100 µL of the appropriate standards and sera. Add 100 µL of Serum Diluent to reagent blank well. Check software and reader requirements for the correct reagent blank well configuration.
4. Incubate each well at room temperature (21 to 25 ºC) for 60 minutes +/- 1 minute.
5. Aspirate or shake out liquid from all wells. If using semi-automated or automated washing equipment add 250-300 µL of diluted Wash Buffer to each well. Aspirate or shake out to remove all liquid. Repeat the wash procedure two times (for a total of three (3) washes) for manual or semi-automated equipment or four times (for a total of five (5) washes) for automated equipment. After the final wash, blot the plate on paper toweling to remove all liquid from the wells.

*IMPORTANT NOTE: Regarding steps 5 and 8 - Insufficient or excessive washing will result in assay variation and will affect validity of results. Therefore, for best results the use of semi-automated or automated equipment set to deliver a volume to completely fill each well (250-300 µL) is recommended. A total of up to five washes maybe necessary with automated equipment. **Complete removal of the Wash Buffer after the last wash is critical for the accurate performance of the test. Also, visually ensure that no bubbles are remaining in the wells.**

6. Add 100 µL Conjugate to each well, including reagent blank well. Avoid bubbles upon addition as they may yield erroneous results.
7. Incubate each well at room temperature (21 to 25 ºC) for 30 minutes +/- 1 minute.
8. Repeat wash as described in Step 5 up to FIVE washes.
9. Add 100 µL Chromogen/Substrate Solution (TMB) to each well, including the reagent blank well, maintaining a constant rate of addition across the plate.
10. Incubate each well at room temperature (21 to 25 ºC) for 1 - 5 minutes.
11. Stop reaction by addition of 50 µL of Stop Solution (1NH2SO4) following the same order of Chromogen/Substrate addition, including the reagent blank well. Tap the plate gently along the outsides, to mix contents of the wells. The plate maybe held up to 1 hour after addition of the Stop Solution before reading.

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

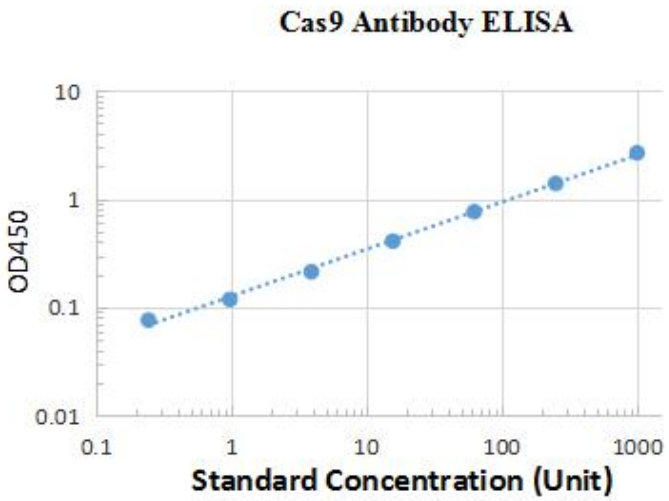
The following figures demonstrate typical results with the Cas9 Host Autoantibody ELISA Kit. One should use the data below for reference only. This data should not be used to interpret actual results.

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 Cas9 Host Autoantibody 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.



Background and More Informations

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. One of the concern is that Cas9 as a foreign and bacterial protein, it may stimulates the host immune system to generate antibodies. The most common subtypes of the antibody are IgM (early but short, appears 1 week after expression or injection and lasts for 2-3 weeks) and IgG (late but long, appears 2 weeks after expression or injection and lasts several months or years). Novateinbio's Cas9 antibody ELISA is designed to be used for both IgM/IgG detection after Cas9 vector/protein is injected into the host. The antibody detection will be customized based on whichever host is used: human, monkey, mouse, bovine, porcine, etc. This is an indirect ELISA for qualitative antibody detection. A semi-quantitative assay kit can also be created based on the customer's preference.

Manufactured and Distributed by:

Novatein Biosciences

310 W Cummings Park,

Woburn, MA, 01801, USA

Phone: (617) 238-1396

Fax: (617) 380-0053

Toll Free: (888) 856-2858

http://www.novateinbio.com/

Email: Info@novateinbio.com