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Cas9 (CRISPR Associated Protein 9) ELISA Kit

Catalog #CAS-MM00B (96 T)

Component Name	Quantity	Component Description	Storage
Pre-coated plate (CAS-MM00B-1)	1 x 96-well plate	96-well plate pre-coated with anti-Cas9 monoclonal antibody.	2-8°C
Cas9 Standard (CAS-MM00B-2)	3 vials	Lyophilized Cas9 standard protein, reconstitute to a concentration of 3.2 µg/mL with 500µl water before use.	
20X Detection Antibody (CAS-MM00B-3)	750µL	Binds to the Cas9 protein in the sample.	
Streptavidin-HRP Conjugate (CAS-MM00B-4)	15mL	Binds to the detection antibody and catalyzes the reaction for color detection.	
10X Assay Buffer (CAS-MM00B-5)	10mL	For diluting standard, sample and detection antibody. Dilute to 1X before use.	
20X Wash Buffer (CAS-MM00B-6)	30mL	20X Wash Buffer containing PBS and Tween-20. Dilute to 1X before use.	
TMB (CAS-MM00B-7)	15mL	Chromogenic substrate for HRP.	
Stop Solution (CAS-MM00B-8)	10mL	0.5M H_2SO_4	

OTHER EQUIPMENT REQUIRED BUT NOT PROVIDED:

- Microplate reader (full wavelength or with 450nm filter)
- Plate washer

ASSAY PERFORMANCE

Detection range: 0.25 ng/mL – 16 ng/mL
Sensitivity: 0.125 ng/mL
Accuracy: CV <10%

STORAGE CONDITIONS AND EXPIRATION DATE

Store the kit components at 2-8°C. The kit is valid for 12 months from the production date. Reconstituted Cas9 standard is recommended to be stored at -80°C and avoid repeated freeze-thaw cycles.

DETECTION PRINCIPLE

This kit uses sandwich ELISA to determine the concentration of Cas9 in the test sample. The capture Cas9 monoclonal antibody is pre-coated on the 96-well plate. Cas9 standard or test sample is added to the pre-coated 96-well plate and will bind to the capture antibody. The biotinylated detection antibody is then added to bind the Cas9-capture antibody complex, followed by the addition of streptavidin HRP conjugate to form the capture antibody-antigen-detection antibody-HRP complex.

The extra detection antibody and the HRP conjugate need to be washed off. The addition of TMB results in color changes. The amplitude of the color change is proportional to the amount of Cas9 that binds to the plate. The reaction is stopped by adding the stop solution and the absorbance is measured at 450nm. The sample Cas9 concentration is calculated from the Cas9 standards titration curve.

OPERATING PROCEDURES

Equilibrate the kit to room temperature before use.

Reagent Preparation:

1. Prepare 1X Wash Buffer (Dilute 20X Wash Buffer with distilled H_2O)

2. Prepare 1X Assay Buffer (Dilute 10X Assay Buffer with distilled H_2O)
3. Prepare 1X Detection Antibody (Dilute the detection antibody with 1X Assay Buffer)

Sample and Standard Preparation:

4. **Preparation of Cas9 standards**: Add 500µL of distilled H_2O to one vial of standard. Dissolve the standard at room temperature for 20 min, mix gently (DO NOT vortex). The final concentration of the Cas9 standard is 3.2 µg/mL. Make the serial dilution of the Cas9 standard following the table below. Two-fold serial dilution of the Cas9 standard with 1X Assay Buffer for the titration curve in duplicates is recommended.

Standard	Concentration (ng/mL)	Dilution
A	16	3 µL Standard in 600µL 1X Assay Buffer
B	8	1:2 dilution from Standard A with 1X Assay Buffer
C	4	1:2 dilution from Standard B with 1X Assay Buffer
D	2	1:2 dilution from Standard C with 1X Assay Buffer
E	1	1:2 dilution from Standard D with 1X Assay Buffer
F	0.5	1:2 dilution from Standard E with 1X Assay Buffer
G	0.25	1:2 dilution from Standard F with 1X Assay Buffer
H (1X Assay Buffer)	0	N/A

5. **Sample preparation**: Dilute the sample with 1X Assay Buffer to make sure the Cas9 concentrations fall within the linear range. If the OD value is outside the range of detection, adjust the dilution factor.

ELISA:

6. **Equilibration**: Take out the 96-well plate, seal unused strips, and immediately return to 4°C. Wash the plate with 300µL 1X wash buffer. Pat dry.
7. **Incubation**: Add the standards and samples to the 96-well plate (100µL per well). Incubate on a shaker (600 rpm) at 37°C for **1 hour**.
8. **Adding detection antibody**: Wash the 96-well plate with 300µL 1X Wash Buffer 3 times. Pat dry and immediately add 1X detection antibody (100 µL per well). Incubate on the shaker (600 rpm) at 37°C for **1 hour**.
9. **Adding Streptavidin-HRP conjugate**: Wash the 96-well plate with 300µL 1X Wash Buffer 3 times. Pat dry and add streptavidin-HRP conjugate (100 µL per well). Incubate on the shaker (600 rpm) at 37°C for **1 hour**
10. **Adding TMB Substrate**: Wash the 96-well plate with 300µL 1X Wash Buffer 3 times. Pat dry and add 100 µL of TMB Substrate to each well. Incubate at 37°C for **10 minutes, protected from light**.
11. **Adding Stop Solution**: Add 50µL of stop solution to each well and mix gently. Immediately read the OD value of each well at 450nm by a microplate reader. It is recommended to read the OD450 values within 5 minutes after adding the stop solution.

DATA ANALYSIS

1. Create a standard curve by plotting the standard concentrations on the x-axis against the OD450 values on the y-axis of a scatterplot. If standards were run in duplicate or triplicate, use the average value. We recommended fitting the data with a 4-parameter logistic fit curve as the standard curve. Other methods such as linear and logarithmic methods may obtain better fitting results and may also be applicable, depending on the specific experimental needs.
2. Calculate the sample Cas9 concentration by entering the sample OD450 value into the equation of the standard curve. If you dilute the sample, multiply by the dilution factor. The lower limit of quantitation (LOQ) is 0.25ng/mL. The sample should be further diluted and retested if the OD450 of the sample falls above the valid linear range.

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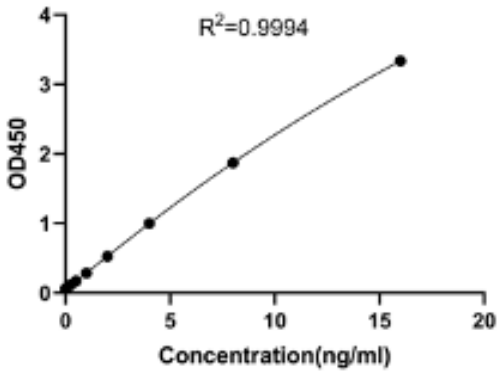
EXAMPLE DATA

Standard	Cas9 Concentration (ng/mL)	OD450
A	16	3.3350
B	8	1.8700
C	4	0.9975
D	2	0.5240
E	1	0.2850
F	0.5	0.1720
G	0.25	0.1165
H	0	0.0613



NOTES

1. The microplate has detachable strips. Do not touch the bottom of the well while disassembling.
2. Do not leave the plate too long after each wash to avoid drying out.
3. 10X Assay Buffer and 20X Wash Buffer may precipitate at 4°C due to high salt concentration. The precipitant can be redissolved at room temperature.
4. Do not use this kit with components from other commercial kits, and do not mix components from different batches of kits. A standard curve must be prepared for each plate, and duplicates are recommended.
5. All reagents must be equilibrated to room temperature (18-25°C) before use. The TMB substrate should be warmed up to 37°C before use.
6. Make sure there is no liquid left in each well after each wash.
7. Using a plate washer can reduce the experimental error. For manual wash, it is recommended to soak the plate in 1X Wash Buffer for 1 minute after each addition.
8. The TMB substrate incubation needs to be protected from light and stopped within 10 minutes.
9. The Stop Solution contains sulfuric acid and may cause irritations on skin or eyes. Rinse immediately with plenty of water and seek medical assistance if necessary.