Datasheet for ABIN5680040

CRISPR-Cas9 ELISA Kit

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Overview

Quantity:	96 tests
Target:	CRISPR-Cas9
Reactivity:	Streptococcus pyogenes
Application:	ELISA

Product Details

Purpose:	Cas9 ELISA Kit is an enzyme immunoassay developed for the detection and quantitation of S. pyogenes Cas9 in cell or tissue lysate samples.
Sample Type:	Cell Samples
Analytical Method:	Quantitative
Detection Method:	Colorimetric
Sensitivity:	1.5 ng/mL
Characteristics:	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. 2
Components:	1. Anti-Cas9 Antibody Coated Plate : One 96-well strip plate (8 x 12). 2. Biotinylated Anti-Cas9 Antibody (1000X) : One 10 µL vial. 3. Streptavidin-Enzyme Conjugate : One 20 µL vial. 4. Assay Diluent : One 50 mL bottle. 5. 10X Wash Buffer : One 100 mL bottle. 6. Substrate Solution : One 12 mL amber bottle. 7. Stop Solution (Part. No. 310808): One 12 mL bottle. Box 2 (shipped on blue ice packs)

Target Details

Target:	CRISPR-Cas9
Alternative Name:	Cas9 (CRISPR Associated Protein 9)
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 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.

Application Details

Plate:	Pre-coated
Reagent Preparation:	• 1X Wash Buffer: Dilute the 10X Wash Buffer to 1X with deionized water. Stir to homogeneity. 3 • 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 5 µg/mL Cas9 Standard Tubes (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
Sample Preparation:	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 4 °C. Assay immediately or store samples at -80 °C for up to three months. Dilute samples in PBS containing 0.1 % BSA as needed.
Assay Procedure:	1. Add 100 µL of Cas9 unknown sample or standard to the Anti-Cas9 Antibody Coated Plate.

Application Details

	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. 4 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. 5
Restrictions:	For Research Use only

Handling

Handling Advice:	Avoid multiple freeze/thaw cycles.
Storage:	4 °C/-80 °C
Storage Comment:	Upon receipt, aliquot and store the Cas9 Standard at -80°C to avoid multiple freeze/thaw cycles. Store all other components at 4°C.