MaxNuclease ELISA Kit

Product Information

Product Name	Catalog Number	Size
MaxNuclease ELISA Kit	NUC-SE00B	96 T

Product Description

Component Number	Component Name	Size	Description	Storage
NUC-SE00B-1	Pre-coated Plate	96 well	Coated with anti-MaxNuclease monoclonal antibody	2-8℃
NUC-SE00B-2	MaxNuclease Protein Standard 0.6 μg/ml (200x)	500 μl/vial	MaxNuclease (lyophilized), dissolve with 500 μl ddH_2O to 0.6 μg /ml before use	
NUC-SE00B-3	HRP-conjugated MaxNuclease Antibody(10x)	1.1 ml/vial	Binds with MaxNuclease and reacts with the substrate	
NUC-SE00B-4	Assay Buffer (10x)	10 ml/bottle	For protein standard and sample dilution	
NUC-SE00B-5	Wash Buffer (20x)	30 ml/bottle	20X Wash Buffer containing PBS, Tween-20, Dilute to 1X before use	
NUC-SE00B-6	TMB	15 ml/bottle	Chromogenic substrate of HRP	
NUC-SE00B-7	Stop Solution	10 ml/bottle	0.5 M H_2SO_4	

OTHER EQUIPMENT REQUIRED BUT NOT PROVIDED:

- Microplate reader (full wavelength or with 450nm filter)
- Plate washer
- Shaking incubator (with temperature control function)
- Pipettes and tips

Assay Performance

(1) Detection Range: 46.88 pg/ml-3000.00 pg/ml

(2) Sensitivity: 23.44 pg/ml

(3) Precision: CV<10%

STORAGE CONDITIONS AND EXPIRATION DATE

Store at 2-8℃ immediately after receiving the kit. Store protein standard solutions at -20℃ or -80℃ for long-term storage, if not used immediately. The shelf life is 12 months starting from manufacturing date.

DETECTION PRINCIPLE

MaxNuclease is a genetically engineered endonuclease derived from *Serratia marcescens* that can degrade all forms of DNAs and RNAs, including double-stranded, single-stranded, circular, or linear RNA and DNA completely to 5'-monophosphate oligonucleotides 2-5 bases in length over a wide range of reaction conditions and is therefore widely used to fully digest and remove nucleic acid residues from biological samples.

This kit uses sandwich ELISA to determine the concentration of MaxNuclease in the test samples. The capture antibody is precoated onto the 96 well plate with intact binding activity with MaxNuclease. After adding in protein standards or testing samples, the maxnuclease will specifically bind to the antibody on the plate. The HRP-conjugated MaxNuclease antibody is added to the plate to form an antibody-Maxnuclease-detecting antibody complex. After rinsing, the HRP substrate TMB is added for chromatic changes. The amplitude of the color change is proportional to the amount of MaxNuclease bound to the plate. Before plate reading, add the stop solution to terminate the reaction. The absorbance is measured at 450nm and the enzyme concentration is calculated from the standard titration curve.

PROCEDURE

(1) Take out the kit from 2-8℃ and warm up to room temperature.

(2) Prepare wash buffer: Dilue 20X wash buffer to 1X with ddH_2O or pure water before use.

(3) Prepare assay buffer: Dilute 10× assay buffer to 1X with ddH_2O or pure water before use.

(4) Prepare HRP-conjugated antibody: Dilute 10×HRP-conjugated antibody with 1X assay buffer before use.

(5) Prepare protein standards: Centrifuge the protein standards at 1000 rpm+ for 30 seconds; this is to accumulate the lyophilized Maxnulcease at the bottom of the vial. Carefully open the lid and add 500 μl ddH_2O to dissolve the powder, making it into a 200× stock buffer (0.6 μg/ml). Add 3 μl 200× protein standards into 597 μl 1× assay buffer, making into a 3 ng/ml MaxNuclease reference standard. Then make a 2-fold serial dilution into 1.5 ng/ml、0.75 ng/ml、0.375

ng/ml、0.187 ng/ml、0.093 ng/ml、0.046 ng/ml, using assay buffer as a blank control. Store the remaining 200X protein standard stock buffer at -20℃ or -80℃.

(6) Prewash: Take out the 96-well plate(s). Add in 300 μl wash buffer and pat dry. Store unused plates in sealed bags at 4-8℃.

(7) Add samples: Add protein standards and samples into the 96 well plates, 100 μl/well. Incubate at 37℃ shaking incubator for 1hr.

(8) Add HRP-conjugated antibody: Wash the plate 4 times, 300 μl/well. Pat dry and then add in HRP-conjugated antibody, 100 μl/well. Incubate at 37℃ shaking incubator for 1hr.

(9) Add TMB substrate: Wash the plate 4 times, 300 μl/well. Pat dry and then add in TMB solution, 100 μl/well. Develop at 37℃ for 10-15 mintues, protected from light. Add 50 μl stop solution in each well to teminate the reaction. Immediately read the OD values under 450 nm within 5 minutes after adding the stop solution.

Data Analysis

(1) Create standard curves

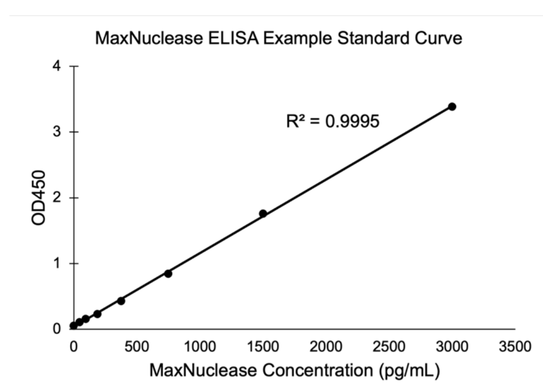
Plot the standard concentrations on the X-axis against OD 450 values on the Y-axis as a catterplot. If standards were run in duplicate or triplicate, use the average value. We recommended fitting the data with a linear curve to use as the standard curve. However, other methods such as a four-parameter algorithm may obtain better fitting results, depending on the specific experimental needs.

(2) Calculate sample concentration

Enter the sample OD450 value into the equation of the standard curve. If samples are diluted, multiply the value with the dilution factor. The lower limit of quantitation (LOQ) is 46.88pg/mL. The sample should be further diluted and retested if the OD450 of the sample falls above the valid linear range.

DATA EXAMPLE

Standard	MaxNuclease Concentration (pg/mL)	OD450
A	3000.00	3.3905
B	1500.00	1.7601
C	750.00	0.8466
D	375.00	0.4314
E	187.50	0.2353
F	93.75	0.1594
G	46.88	0.1123
H	0.00	0.0542



NOTES

(1) The microplate has detachable strips. Do not touch the bottom of the well while disassembling. Do not leave the plate too long after each wash to avoid the plates drying out.

(2) It is normal that 10X Assay Buffer and 20X Wash Buffer may precipitate at 4°C due to high salt concentration. The precipitates can be redissolved at room temperature.

(3) 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.

(4) All reagents must be equilibrated to room temperature (18-25°C) before use. The TMB substrate should be warmed to 37°C before use.

(5) Make sure there is no liquid left in each well after each wash.

(6) 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.

(7) The Stop Solution contains sulfuric acid and may cause skin or eye burn. Rinse immediately with plenty of water and seek medical assistance if necessary.

(8) To ensure accurate results, the order of adding samples in each step should be consistent to reduce experimental errors caused by sample addition problems, so that the reaction time and color development time for each well are consistent.

(9) For research use only