Universal Nuclease ELISA Kit

Please read the manual carefully before use.

Cat. No. NE110

Storage: 1 year in the dark at 2-8°C

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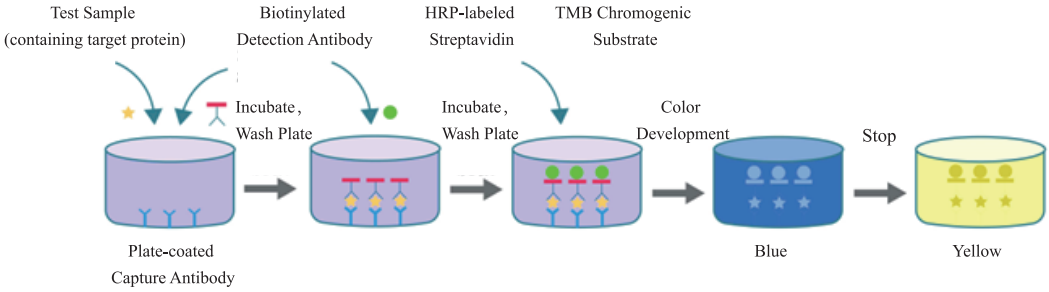
Description

Universal Nuclease (Benzonase®) is a non-specific, broad-spectrum endonuclease derived from Serratia marcescens. It is produced through genetic engineering, expressed in Escherichia coli, and subsequently purified. Also known as a non-restriction endonuclease or broad-spectrum nuclease, this enzyme cleaves DNA and RNA strands indiscriminately at any nucleotide site, degrading nucleic acids into 5'-monophosphorylated oligonucleotides 2-5 bases in length. Consequently, Universal Nuclease efficiently degrades all forms of DNA and RNA (double-stranded, single-stranded, circular, or linear) without causing protein hydrolysis. It is widely used to remove nucleic acid residues and contaminants from biological products.

This kit employs a sandwich ELISA (Enzyme-Linked Immunosorbent Assay) method for the quantitative determination of Universal Nuclease residues in biological products. The microplate is pre-coated with a high-affinity Universal Nuclease capture antibody. Both standards/samples and a biotin-labeled Universal Nuclease detection antibody are added simultaneously to the wells. During incubation, any Universal Nuclease present in the sample binds specifically to both the immobilized capture antibody on the plate and the biotinylated detection antibody. After a wash step to remove unbound substances, Streptavidin conjugated to Horseradish Peroxidase (Streptavidin-HRP) is added and incubated. The high-affinity, non-covalent interaction between biotin on the detection antibody and streptavidin forms an "capture antibody - Universal Nuclease - detection antibody - Streptavidin-HRP" immune complex. Following another wash, the chromogenic substrate TMB is added to the wells. The HRP enzyme catalyzes the conversion of TMB into a blue product. The intensity of this colorimetric reaction is directly proportional to the concentration of Universal Nuclease in the sample. A stop solution is then added to terminate the reaction, and the absorbance is measured at 450 nm (with a reference wavelength of 570-630 nm). By constructing a standard curve from the absorbance values of the standards, the concentration of Universal Nuclease in the samples can be determined. This kit offers high specificity, superior sensitivity, and a more convenient operational procedure.



Schematic Diagram of Sandwich ELISA Principle

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Kit Contents

Component	NE110-01	Storage
Universal Nuclease Antibody Pre-coated Microplate	96 T	2~8°C
Universal Nuclease Standard (100 ng/ml)	120 µl/tube	2~8°C
100× Universal Nuclease Detection Antibody	60 µl/tube	2~8°C
Standard, Sample & Antibody Diluent	25 ml/bottle	2~8°C
100× Streptavidin-HRP	120 µl/tube	2~8°C (Protect from light)
Streptavidin-HRP Diluent	15 ml/bottle	2~8°C
20× Wash Buffer	50 ml/bottle	2~8°C
TMB Chromogenic Substrate	12 ml/bottle	2~8°C (Protect from light)
Stop Solution	6 ml/bottle	2~8°C
Plate Sealer	4 pcs	

Note: When the kit has been opened, it can be stored at 2~8°C for 1 month. Unopened kits should be used within the 1-year shelf life.

Materials and Equipment Required but Not Provided

1. Microplate Reader: Primary wavelength 450 nm, reference wavelength 620 nm.
2. Deionized Water.
3. Laboratory consumables and equipment required for the procedure, such as microcentrifuge (EP) tubes, pipettes, pipette tips, and measuring cylinders.
4. Microplate shaker.
5. Automatic microplate washer, or an 8-channel manual wash bottle, or a multi-channel pipette.

Preparation of Working Solutions

Equilibrate all reagents to room temperature before use.

1) **1× Universal Nuclease Detection Antibody**: Briefly centrifuge the vial before use to collect the liquid at the bottom. According to the amount required for the current experiment, dilute the 100× Universal Nuclease Detection Antibody to a 1× working concentration using the Standard, Sample & Antibody Diluent. The prepared 1× detection antibody must be used within 15 minutes.
2) **1× Streptavidin-HRP:** Briefly centrifuge the vial before use to collect the liquid at the bottom. According to the amount required for the current experiment, dilute the 100× Streptavidin-HRP to a 1× working concentration using the Streptavidin-HRP Diluent. The prepared 1× Streptavidin-HRP must be used within 15 minutes.
3) **1× Wash Buffer:** According to the amount required for the current experiment, dilute the 20× Wash Buffer to a 1× working concentration using deionized water. The prepared 1× Wash Buffer can be stored at 2~8°C for up to 30 days.
4) **Standard Curve Preparation:** Perform a 10-fold initial dilution of the standard: Add 60 µl of the standard to 540 µl of Standard, Sample & Antibody Diluent. Mix thoroughly. Label this as Tube #1 (concentration: 10 ng/ml). Then, perform a series of 2-fold serial dilutions as shown in the diagram below: Add 300 µl of Standard, Sample & Antibody Diluent to Tubes #2 through #7. Transfer 300 µl from Tube #1 to Tube #2 and mix thoroughly (concentration: 5 ng/ml). Transfer 300 µl from Tube #2 to Tube #3 and mix thoroughly (concentration: 2.5 ng/ml). Continue this serial dilution through Tube #7 (concentration: 0.16 ng/ml). The 10 ng/ml concentration (Tube #1) serves as the highest point of the standard curve. The Standard, Sample & Antibody Diluent serves as the zero standard (0 ng/ml), i.e., the blank.

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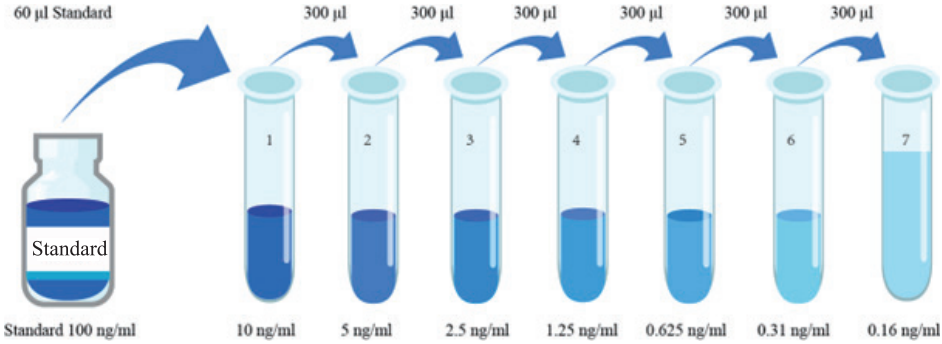

Test Sample Preparation

Dilute the sample according to a certain dilution factor. The specific dilution factor for the sample needs to be determined by spiking the sample and evaluating the spike recovery rate and dilution linearity to identify an appropriate dilution factor.

Note: The sample pH should be maintained between 6.0 and 8.0. Excessively low or high pH may lead to abnormal measurement values.

Assay Procedure

1. Before performing the assay, equilibrate all reagents to room temperature. Take out the required number of microplate strips for the current experiment. Immediately reseal the unused strips in the aluminum foil bag and store them at 2–8°C.
2. Add the diluted standards and prepared samples to the appropriate wells. Add Standard & Sample & Antibody Diluent to the blank well. Add 100 µl per well. It is recommended to test both standards and samples in duplicate, and the order of adding reagents should be consistent to ensure uniform results across replicates.
3. Add 50 µl of 1× Detection Antibody to each well. Mix by shaking the plate on a microplate shaker for 30 seconds. Cover the plate with a sealing film and incubate at 37°C for 1.5 hours.
4. Discard the liquid from the wells. Wash the plate using 1× Wash Solution, adding 300 µl per well for each wash. It is recommended to shake the plate on a microplate shaker for 30 seconds before discarding the wash solution during each wash cycle. Repeat the wash process 5 times. After the final wash, blot the plate dry on absorbent paper.
5. Add 100 µl of 1× Streptavidin-HRP to each well. Mix by shaking the plate on a microplate shaker for 30 seconds. Cover the plate with a sealing film and incubate at 37°C for 30 minutes.
6. Repeat Step 4.
7. Add 100 µl of TMB Substrate to each well. Mix by shaking the plate on a microplate shaker for 30 seconds. Cover the plate with a sealing film and incubate at room temperature for 20 minutes.
8. After incubation, add 50 µl of Stop Solution to each well. Read the plate immediately at a primary wavelength of 450 nm and a reference wavelength of 620 nm.
9. After the experiment, return any unused reagents and the microplate frame to the kit and store at 2–8°C. It is recommended to use the remaining components within one month.

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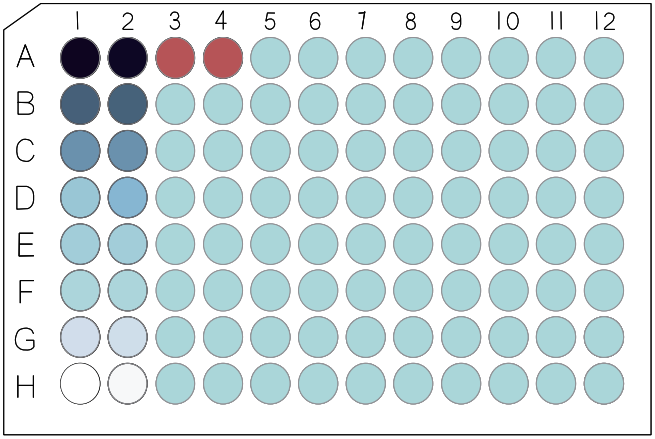
Microplate Well Loading Diagram



Notes: A1/A2：100 µl 10 ng /ml标准品
B1/B2：100 µl 5 ng /ml Standard
C1/C2：100 µl 2.5 ng /ml Standard
D1/D2：100 µl 1.25 ng/ml Standard
E1/E2：100 µl 0.625 ng/ml Standard
F1/F2：100 µl 0.31 ng/ml Standard
G1/G2：100 µl 0.16 ng/ml Standard
H1/H2：100 µl 0 ng/ml Standard(Standard & Sample & Antibody Diluent)
A3/A4：100 µl Sample

Result Analysis

1. Perform a dual-wavelength measurement using the microplate reader, determining the OD values at the primary wavelength (450 nm) and the reference wavelength (620 nm). The final OD value for each well is calculated by subtracting the measured OD at 620 nm from the measured OD at 450 nm.
2. Calculate the average OD value for the duplicate standard wells. Then, subtract the blank value (the average OD value of the 0 ng/ml standard) to obtain the corrected OD value for each standard. Generate the standard curve using linear regression or a four-parameter logistic (4PL) curve fit, with the standard concentration on the x-axis and the corrected OD value on the y-axis.
3. Calculate the sample concentration using the sample's OD value and the standard curve equation. If a sample's OD value exceeds the upper limit of the standard curve, the sample should be appropriately diluted and re-tested. The final concentration must then be calculated by multiplying the result by the corresponding dilution factor.

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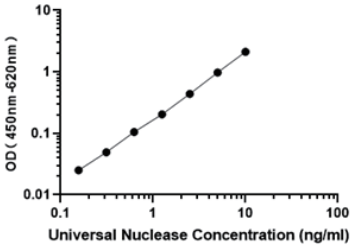
Reference Data

A standard curve must be generated for each assay. The data provided below is for illustrative purposes only.

Standard ng/ml	Average OD Value	Corrected Value
10	2.145	2.118
5	1.002	0.975
2.5	0.464	0.437
1.25	0.231	0.204
0.625	0.132	0.105
0.31	0.076	0.049
0.16	0.052	0.025
0	0.027	0



Precision

Intra-assay Precision

Intra-assay precision is assessed by measuring 3 samples of known concentrations in 20 replicates on the same microplate.

Inter-assay Precision

Inter-assay precision is assessed by measuring 3 samples of known concentrations in 20 replicates across different microplates.

	Intra-assay			Inter-assay		
	1	2	3	1	2	3
Mean (pg/ml)	7.9	2.2	0.56	6.7	1.9	0.58
Standard Deviation	0.14	0.03	0.02	0.13	0.04	0.01
CV (%)	1.8	1.5	3.2	2.0	2.4	2.4

Sensitivity

The minimum detectable concentration of Universal Nuclease is 41.7 pg/ml. The sensitivity is calculated as the concentration corresponding to the mean OD value of 20 replicate zero standards plus two times the standard deviation.

Precautions

1. This kit should be stored at 2~8°C in the dark and used up within 1 month after opening.
2. To ensure accurate results, a standard curve is required for each assay.
3. All reagents used in the experiment should be thoroughly mixed.
4. After each plate washing, pat dry on a paper towel. If there are air bubbles in the plate wells, use a pipette tip to puncture them. Note that only one pipette tip can be used in each well to avoid cross-contamination.
5. TMB chromogenic substrate is a colorless and transparent liquid, please do not use it if there is discoloration.
6. After TMB develops color, it can be judged whether it is necessary to add a stop solution in advance or later according to the depth of color development.
7. After adding the stop solution, read within 30 minutes.

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8. It is recommended to use the main wavelength of 450 nm and the reference wavelength of 620 nm for reading. If only a single wavelength of 450 nm is used for reading, the overall OD value may be high, and the blank value will also increase accordingly, resulting in a decrease in the accuracy of the kit.
9. Personal protective equipments are necessary in experiments for safety reasons. The stop solution in the kit is corrosive. Take care when using the reagent to avoid the risks. In case of accidental contact, please rinse with plenty of water and seek medical attention in time.
10. To avoid cross-contamination, use a new disposable pipette tips for each transfer. Please use disposable test tubes, pipette tips, plate sealers and clean plastic containers in the experiment.
11. Kit components from different batches or different sources cannot be used in combination.

For research use only, not for clinical diagnosis.

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