Ver.EN20230913

UCF.ME™ UltraNuclease ELISA Kit

Product description

UltraNuclease, also known as non-restrictive endonuclease, broad-spectrum nuclease, is a non-specific endonuclease derived from Serratia Marcescen, which hydrolyzes internal phosphodiester bonds between any nucleotides in nucleic acids to produce 5'-monophosphate oligonucleotides of 2-5 bases in length. It can degrade DNA and RNA in various forms (double-stranded, single-stranded, linear, circular, native or denatured) under a very broad range of conditions (6 M Urea, 0.1 M Guanidine HCl, 0.4% Triton X-100, 0.1% SDS, 1 mM EDTA, 1 mM PMSF) and is widely used to remove nucleic acids from biological products. UltraNuclease can also be removed by corresponding methods subsequently.

This kit uses the principle of double-antibody sandwich enzyme-linked immunosorbent assay (sandwich ELISA) to detect the residues of denatured and non-denatured UltraNuclease. First, coat the microplate with anti-UltraNuclease rabbit polyclonal antibody to form a solid-phase antibody. Second, add UltraNuclease standard and test sample to the solid-phase antibody microplate, then add biotin labeled Anti-UltraNuclease polyclonal antibody, and finally add horseradish peroxidase-labeled streptavidin (SA-HRP) to form an antibody + antigen + antibody-Biotin + SA-HRP complex. Subsequently, add TMB substrate into the complex to observe color reaction after washing the complex. TMB is converted into blue under the catalysis of HRP enzyme and finally converted into yellow in the presence of acid, and the shade of color is positively correlated with the amount of UltraNuclease in the sample.

The detection range of this kit is 0.047-3 ng/mL; the lower detection limit is 23.5 pg/mL.

Specifications

Cat.No.	36718ES59
Size	96 T

Components

Components No.	Name	36718ES59
36718-A	ELISA Microplates	1 Plate
36718-B	Detection Antibody：Biotin-conjugated Rabbit Anti-UltraNuclease Antibodies	35 μL (0.2 mg/mL)
36718-C	Standard: UltraNuclease	1 vial (500 ng/mL)
36718-D	HRP-conjugated Streptavidin	10 μL
36718-E	Dilution Buffer 1	45 mL
36718-F	20×Wash Buffer	50 mL
36718-G	Dilution Buffer 2	30 mL
36718-H	TMB	15 mL
36718-I	Sealing Film	5 pieces

* The stop solution is not provided in this kit, it need to be prepared by user. 1M HCl can be used as the stop solution.

Storage

This product should be stored at 2°C ~8°C. Unopened product is valid for one year. Once the reagent is opened, it is valid for half a year. **Never freeze this product.**

*1. The diluted washing buffer and dilution buffer can be stored at 2°C ~8°C for 1 week.

*2. Standard 36701-C is liquid and stored at 2°C ~8°C for 1 year.

*3. Prepared detection antibody and stop buffer can be stored at 2°C ~8°C for 1 month.

Instructions

1. Preparations before the experiment

1) Materials required but not provided:

a. Graduated cylinder, Beaker, Centrifuge tube of different volumes;

b. Dust-free paper (used for plate washing)

c. Sterile pipette tips;

d. Pre-treated plates;

e. Deionized water or double distilled water.

2) Instruments and equipment required but not provided:

a. 37°C incubator;

b. Plate washer;

c. Timer, Vortex and Centrifuge;

d. Single channel or multi-channel pipettes

e. 2°C ~8°C refrigerator;

f. Microplate reader (such as Molecular Devices: M and i series) which with can measure the absorbance at 450 nm.

2. Assay method

1) Reagent preparation

All reagent components and samples to be tested need be balanced to the room temperature before use and be prepared freshly for use.

a. Preparation of 1×Wash Buffer:

Equilibrate the concentrated solution to room temperature and dissolve it fully without crystals. Mix well and transfer 50 mL of **20× Wash Buffer** into container, and then bring the volume to 1 L with ultrapure water. The specific volume can be prepared according to the amount used each time.

b. Preparation of Detection Antibody:

Centrifuge at 10,000 rpm for 20s before use, and then dilute the detection antibody to a working solution concentration of 0.5 μg/mL with **Dilution Buffer 2**.

c. Preparation of HRP-conjugated Streptavidin:

Centrifuge at 10,000 r for 20s before use, and then dilute the HRP-conjugated Streptavidin to the working concentration by 1:5000 dilution with **Dilution buffer 2**.

d. **Preparation of Stop solution:**

Add 10mL concentrated HCl into 110 mL purified water and mix well, then get the 1M HCl. The prepared 1M HCl can be stored at 2°C ~8°C for 1 year.

2) Standard curve preparation:

Prepare 8 clean 1.5 mL centrifuge tubes and label them sequentially according to the standard concentration. Pipette 994 μL of **Dilution Buffer 1** to the centrifuge tube marked with 3 ng/mL, and pipette 500 μL to the other 7 tubes. Transfer 6 μL of 500 ng/mL UltraNuclease Standard to the centrifuge tube marked 3 ng/mL and mix well, then transfer 500 μL to the centrifuge tube with the next labeled concentration, mix well, and perform a series of 2-fold gradient dilutions of the standard. It can be carried out according to the following table and the initial maximum concentration is 3 ng/mL, the minimum is 0.047 ng/mL. A corresponding standard curve needs to be prepared for each test, and standard curves of different kits and different experiments cannot be mixed. For sample testing, the required standard volume for each well is 100 μL. Note that the preparation volume should be higher than the required volume to avoid insufficient volume.

Vial	Dilution Buffer 1 (μL)	UltraNuclease Standard and volume (μL)	Standard final concentration (ng/mL)
A	994	6 (500 ng/mL)	3
B	500	500 A	1.5
C	500	500 B	0.75
D	500	500 C	0.375
E	500	500 D	0.188
F	500	500 E	0.094
G	500	500 F	0.047
H	300	0	0

Table 1. UltraNuclease standard preparation

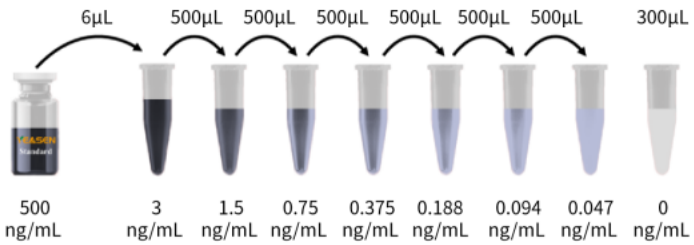

Figure 1. Simplified flow chart of standard configuration

3) Test samples preparation:

Dilute the test samples with certain dilution factor by using **Dilution Buffer 1**. The specific dilution factors of samples need to be evaluated with suitable dilution linearity and spike recovery.

4) Experiment flow

All reagent components and samples to be tested need be balanced to the room temperature before use. It is strongly recommended that all standards and test samples be assayed in duplicate.

a. Reagents and samples preparation:

Prepare required reagents, diluted standards and samples.

b. Strip Determination:

Calculate the strips required for test samples and standards, take strips from the foil bag, put the remaining strips back into the foil bag, seal the bag, and store at 2°C ~8°C.

c. ELISA plate washing:

Wash the plate for 3 times with 1×Wash Buffer (300 μL/well), and pat the ELISA plate dry. Washing the plate has a major impact on the test results, make sure that no wash solution remains in the plate.

d. Samples incubation:

Add standards and test samples, 100 μL/well, ensure that the sample adding is completed within 15 minutes, and incubate at 37°C for 1 h.

e. ELISA plate washing:

Discard the liquid in the wells, wash the plate with 1×Wash Buffer (300 μL/well) for 5 times, and pat the ELISA plate dry.

f. Detection antibody incubation:

Add the pre-diluted detection antibody into the ELISA plate, 100 μL/well, and incubate at 37°C for 1 h.

g. ELISA plate washing:

Discard the liquid in the wells, wash the plate with 1×Wash Buffer (300 μL/well) for 5 times, and pat the ELISA plate dry.

h. HRP-conjugated Streptavidin incubation:

Add the pre-diluted HRP-conjugated Streptavidin into the ELISA plate, 100 μL/well, and incubate at 37°C for 40 min.

i. ELISA plate washing:

Discard the liquid in the well, wash the plate with 1×Wash Buffer (300 μL/well) for 5 times, and pat the ELISA plate dry.

j. Color reaction:

Balance the TMB solution to room temperature 10 minutes before use, add the TMB to the ELISA plate, 100 μL/well, and incubate at 37°C for 15 minutes away from light.

k. Stopping:

Add stop solution to the plate, 50 μL/well, and shake the plate gently until the color is uniform.

l. Reading:

Read the absorbance at 450 nm within 20 min.

5) Result Analysis

a. If the OD value of the sample exceeds the maximum peak of the standard curve, the sample should be diluted and re-measured.

b. Standard curve drawing: ELISACalc.exe Regression analysis/Curve fitting software is recommended to draw the standard Curve (X-standard concentration ng/mL; Y-final OD450 nm) by regression of a conic fitting according to the absorbance of the UltraNuclease standard (subtracting the OD value of the standard blank well namely the final reading, or not subtracting the OD value of the blank well). Calculate the UltraNuclease concentration in the sample based on the standard curve and the dilution folds of the sample.

c. The typical Standard Curve is as follows (the following standard curve is for reference only, and the sample content should be calculated based on the standard curve generated in the same experiment) :

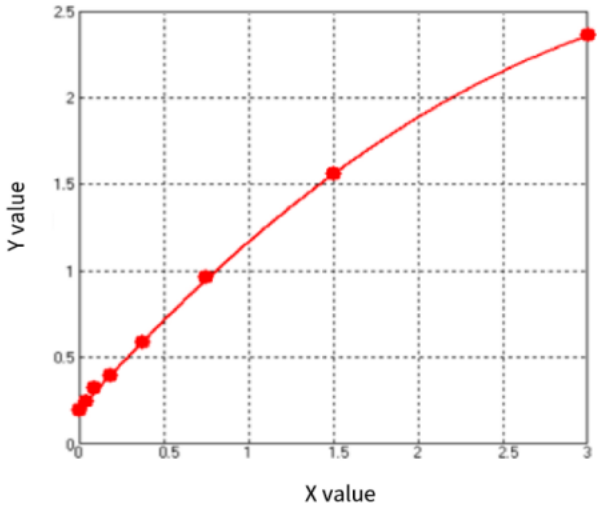

Figure 2 Typical standard curve

Concentration (ng/mL)	OD450 Value
3	2.359
1.5	1.559
0.750	0.960
0.375	0.585
0.188	0.397
0.093	0.326
0.047	0.242
0.023	0.188
0	0.063

Table 2 Typical standard curve data

6) Experiment flow chart

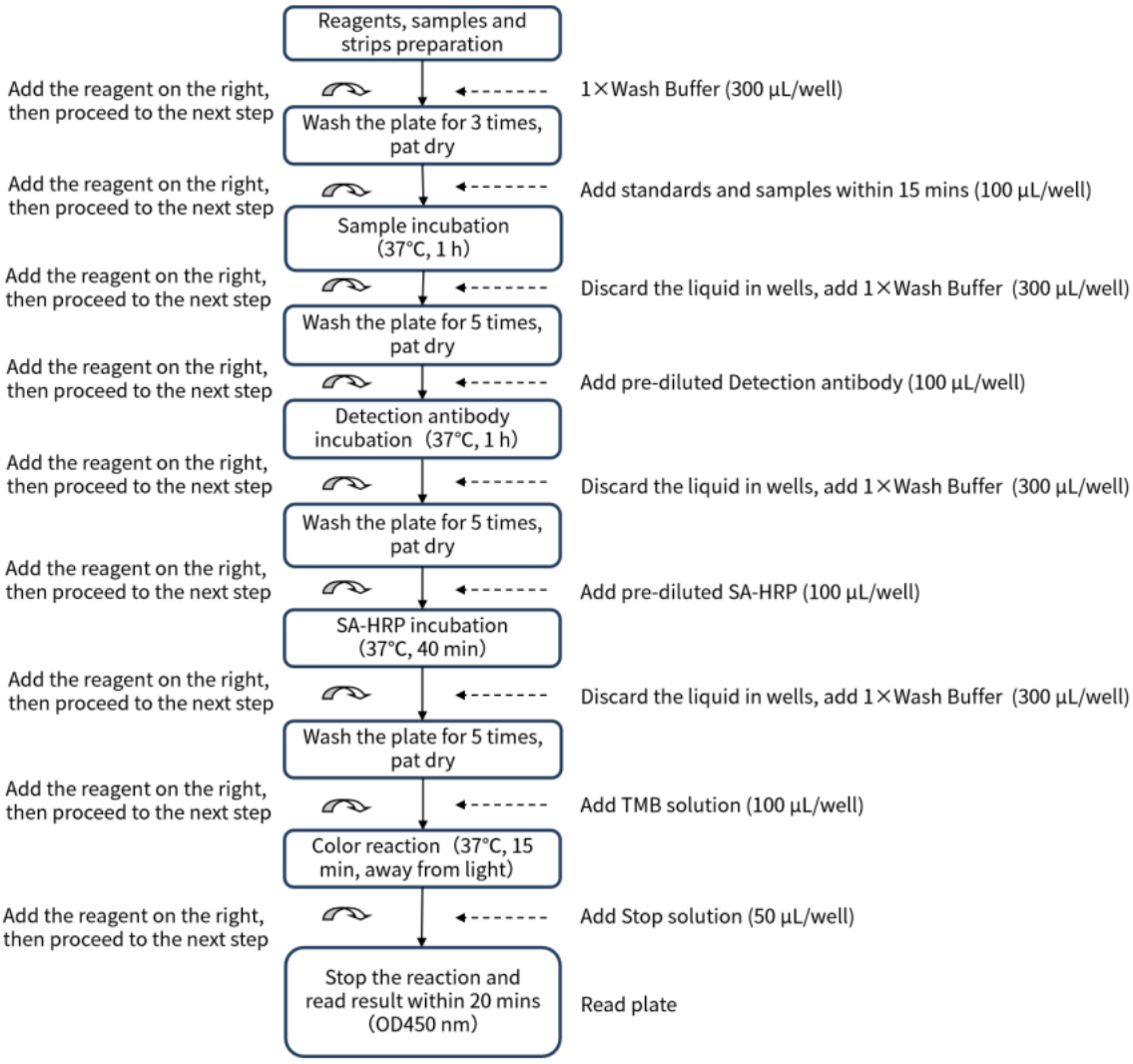

Figure 3 Experiment flow chart

Notes

1. Please read this manual carefully before using this kit. The kit needs to be used up within its shelf life.
2. Mixing of related reagents from different batches is prohibited.
3. The experiment should be conducted in a standardized manner, vortex and mix well for each reagents and samples before use.
4. The kit is designed for detecting the target antigens and samples marked in the instructions only. Other applications need to be designed and verified by the user, and the reliability and accuracy should be evaluated based on the results.
5. For your safety and health, please wear personal protective equipment (PPE), such as laboratory coats and disposable gloves, when operating with this product.
6. This product is for research use only.