Salt Active UltraNuclease ELISA Kit

Product description

Salt Active UltraNuclease is a non-specific endonuclease which derived from Marine microorganisms and is expressed and purified in Escherichia coli (E. coli) by genetically engineered. This nonspecific, recombinant endonuclease has optimum activity at high salt concentration (500mM NaCl), which can improve efficiency and yield in various workflows.

This kit uses the principle of double-antibody sandwich enzyme-linked immunosorbent assay (sandwich ELISA) to detect the residual Salt Active UltraNuclease in samples. First, add the Salt Active UltraNuclease standard and test sample to the Anti-Salt Active UltraNuclease coated microtiter strips (36719-A), then add the diluted biotin labeled Anti-UltraNuclease antibody (36719-C), and finally add Streptavidin-HRP (36719-D) to form an antibody + antigen + antibody-Biotin + SA-HRP complex. Subsequently, add TMB substrate (36719-H) 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 Salt Active UltraNuclease in the sample.

The detection range of this kit is 0.047-3 ng/mL; the lower detection limit is 0.024 ng/mL.

Specifications

Cat.No.	36719ES48 / 36719ES96
Size	48 T / 96 T

Components

Components No.	Name	36719ES48	36719ES96
36719-A	Anti-Salt Active UltraNuclease coated microtiter strips	48 T	96 T
36719-B*	Standard: Salt Active UltraNuclease	1 vial	2 vial
36719-C	Detection Antibody：Biotin-conjugated Antibodies	60 μL	120 μL
36719-D	Streptavidin-HRP	30 μL	60 μL
36719-E	Dilution Buffer 1	25 mL	50 mL
36719-F	Wash Buffer Concentrate (20×)	25 mL	50 mL
36719-G	Dilution Buffer 2	15 mL	30 mL
36719-H	TMB Substrate	8 mL	15 mL
36719-I	Plate Sealer	3 each	5 each

* 1. 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;

* 2. The Standard (36719-B) is Lyophilized powder.

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.

*Upon receipt of the kit, please check whether all components are complete and immediately store them in corresponding condition.

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 (also with the reference wavelength at 630nm).

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 Wash Buffer Concentrate (20×) to room temperature and dissolve it fully without crystals. Then dilute it by 1:20 with the the Deionized water or double distilled water according the requirement. For example, mix well and transfer 50 mL of Wash Buffer Concentrate (20×) into container, and then bring the volume to 1 L with Deionized water or double distilled water. If crystallization occurs in Wash Buffer Concentrate (20×), warm it in a water bath at 50°C until the crystallization completely disappears.

b. Preparation of Detection Antibody:

Centrifuge at 10,000 rpm for 20s before use, and then dilute the detection antibody by 1: 100 with **Dilution Buffer 2** to make a working solution.

c. Preparation of Streptavidin-HRP:

Centrifuge at 10,000 r for 20s before use, and then dilute the Streptavidin-HRP by 1: 500 with **Dilution buffer 2** to make a working solution.

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. Take

1 vial lyophilized standard and dissolve the standard powder with Dilution buffer 1 marked in volume on the tube. Let the tube stand for about 10 minutes and gently mix well, the final concentration is 6 ng/mL.

Pipette 300μL of Dilution Buffer 1 to each pre-marked centrifuge tubes, then transfer 300μL 6 ng/mL dissolved standard into the tube which marked with 3 ng/mL, then mix well. Subsequently, transfer 300 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.

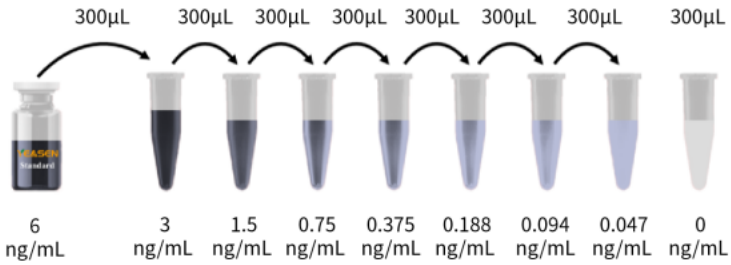

Figure 1 Simplified flow chart of standard configuration

Vial	Volume of Dilution Buffer 1 (μL)	Volume of Standard (μL)	Final concentration of Standard (ng/mL)
A	300	300 (6 ng/mL)	3
B	300	300 A	1.5
C	300	300 B	0.75
D	300	300 C	0.375
E	300	300 D	0.188
F	300	300 E	0.094
G	300	300 F	0.047
H	300	0	0

Table 1 Salt Active UltraNuclease standard preparation

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. The plate washing has an important 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/650 nm within 20 min. 450 nm as the detection wavelength, and 650 nm as the reference wavelength.

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:

The standard curve is drawn with the concentration of the standard as the abscissa and the absorbance value of OD450nm-630nm as the ordinate. A variety of mapping and statistical software can be used to aid in drawing standard curves and calculating unknown sample concentrations. The four-parameter fitting method often has better curve fitting effect, and other methods such as linear and double-logarithm method may also obtain better fitting results, which need to be analyzed according to specific experimental data. Finally, the residual Salt Active UltraNuclease concentration in the sample will be calculated according to the standard curve and the dilution ratio 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) :

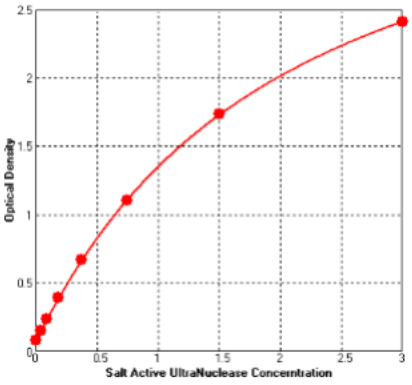

Figure 2 Typical standard curve

Standard Conc. (ng/mL)	OD		Mean OD	Calibration value
3.000	2.411	2.412	2.412	2.414
1.500	1.716	1.760	1.738	1.731
0.750	1.089	1.116	1.103	1.113
0.375	0.653	0.687	0.670	0.666
0.188	0.385	0.400	0.393	0.391
0.094	0.236	0.245	0.241	0.237
0.047	0.148	0.159	0.154	0.159
0.000	0.083	0.080	0.082	/

Table 2 Typical standard curve data

6) Experiment flow chart

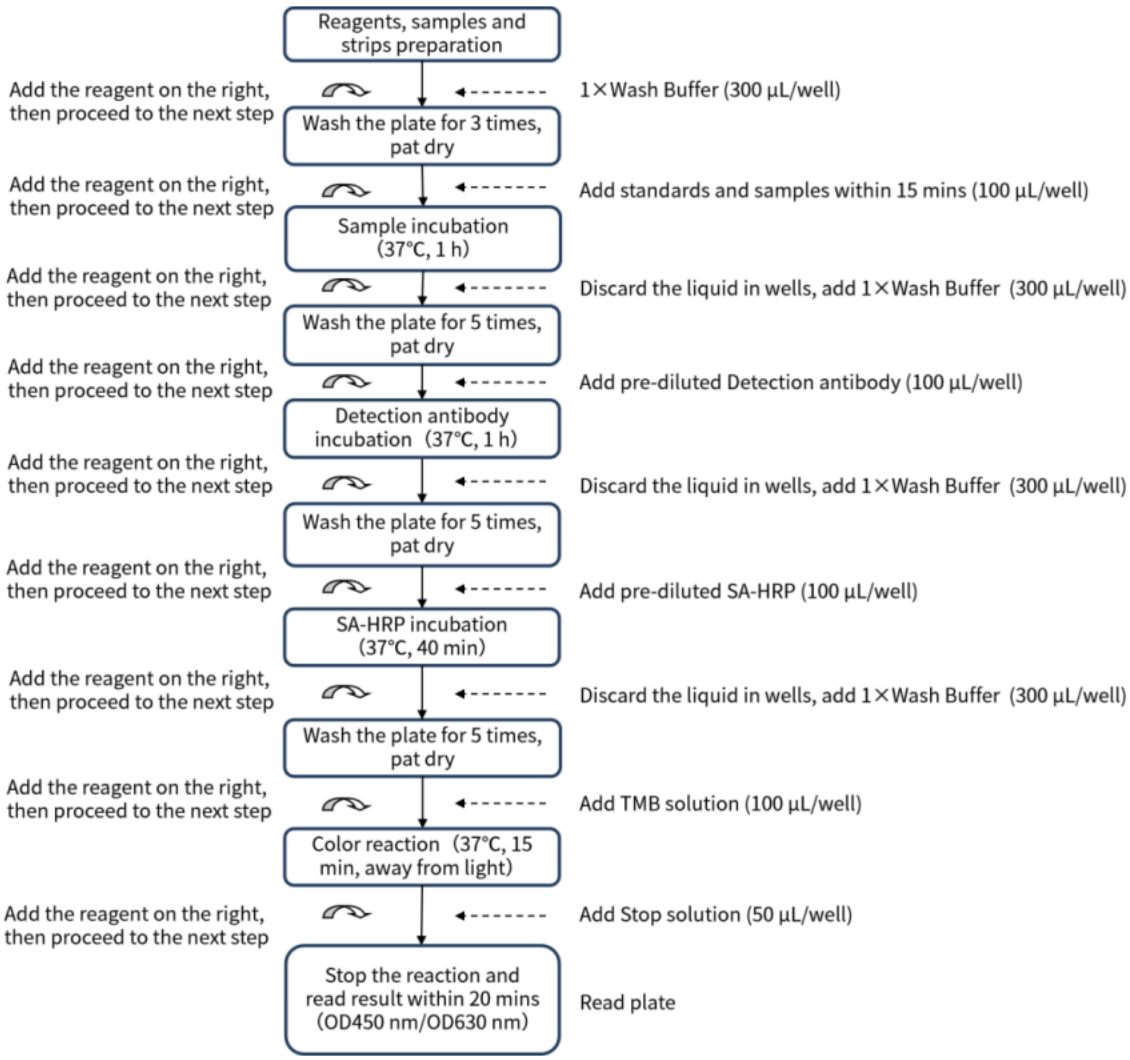

Figure 3 Experiment flow chart

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.