Version 22-04/24

User's Manual

DNase I ELISA Kit

REF **DEIA-BY008**

Σ **96T**

RUO

This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

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PRODUCT INFORMATION

Intended Use

For rapid determination of DNase I content in samples.

Principles of Testing

This kit was based on sandwich enzyme-linked immune-sorbent assay technology. Capture antibody was pre-coated onto 96-well plates. The standards, test samples and detection antibody were added to the wells subsequently, and washed with wash buffer. Streptavidin HRP was added and unbound conjugates were washed away with wash buffer. TMB substrates were used to visualize HRP enzymatic reaction. TMB was catalyzed by HRP to produce a blue color product that changed into yellow after adding acidic stop solution. The density of yellow is proportional to the target amount of sample captured in plate. Read the O.D. absorbance at 450nm/630nm in a microplate reader, and then the concentration of target can be calculated.

Reagents And Materials Provided

1. DNase I Coated Plate: 8×12, Ready-to-use;
2. Detection antibody: 150μL×1, 1:100 diluted with Antibody Diluent Buffer;
3. Streptavidin HRP Conjugate: 150μL×1, 1:100 diluted with Enzyme Conjugate Diluent Buffer;
4. DNase I Standard: 30μL×1 (200μg/mL), dilute according to the recommended method;
5. Sample Diluent Buffer: 60mL×1, Ready-to-use;
6. Antibody Diluent Buffer: 12mL×1, Ready-to-use;
7. Enzyme Conjugate Diluent Buffer: 12mL×1, Ready-to-use;
8. 20× PBST Wash Buffer: 50mL×1, 1:20 diluted with ddH_2O;
9. TMB Substrate: 11mL×1, Ready-to-use;
10. Stop Solution: 7mL×1, Ready-to-use;
11. Plate Sealer: 5 pieces
12. Datasheet: 1 copy

Materials Required But Not Supplied

1. Microplate reader
2. Microplate thermostatic oscillator
3. Vortex mixer
4. Precision single and multi-channel pipette and disposable tips
5. Clean tubes and Eppendorf tubes
6. New filter paper
7. Deionized or distilled water

Storage

Detection antibodies, Enzyme conjugates and Standards should be stored at -20°C, and the other components should be stored at 2-8 °C in the dark. The validity period is 12 months.

Reagent Preparation

Bring all reagents and samples to room temperature (18-25°C) for 30 minutes before use.

1. 1× PBST Wash Solution:

Dilute the contents of each vial of the 20× PBST Wash Buffer with distilled or deionised water to a final volume of 1000 mL prior to use.

2. Preparation of Detection Antibody Working Solution:

Calculate required total volume of the working solution. Dilute the Detection antibody with Antibody Diluent Buffer at 1:100 and mix them thoroughly.

3. Preparation of Streptavidin HRP Conjugate Working Solution:

Calculate required total volume of the working solution. Dilute the Streptavidin HRP with Enzyme Conjugate Diluent Buffer at 1:100 and mix them thoroughly.

4. Standards:

No.	Standard solution concentration (ng/mL)	Volume of standard solution (μL)	Volume of sample diluent buffer (μL)	Total volume (μL)	Final concentration (ng/mL)	Residual volume (μL)
Pre-1	200000	5	495	500	2000	436
Pre-2	2000	64	936	1000	128	700
8	128	300	300	600	64	300
7	64	300	300	600	32	300
6	32	300	300	600	16	300
5	16	300	300	600	8	300
4	8	300	300	600	4	300
3	4	300	300	600	2	300
2	2	300	300	600	1	600
1	/	/	300	300	0	300

Assay Procedure

When diluting samples and reagents, they must be mixed completely and evenly to avoid bubbles.

1. Determine the required number of strips according to the number of test wells, and put the remaining strips back into the aluminum foil bag containing desiccant and seal it.
2. Pipette 100 μl of standard, test samples and negative control into the wells. Seal the plate with a cover and incubate at 37°C for 60 minutes. (Note: Orbital shaking at 200-300 rpm is recommended for the duration of the incubation.)
3. Wash: Remove the cover and discard the plate content, and wash plate 3 times with 1× PBST Wash Buffer (300μl/well). Pat dry on filter paper after each wash. Do NOT let the wells dry completely at any time.
4. Add 100ul Detection antibody working solution into above wells (standard, test sample and control wells).

Add the solution at the bottom of each well without touching the sidewall, cover the plate and incubate at 37°C for 60 minutes. (Note: Orbital shaking at 200-300 rpm is recommended for the duration of the incubation.)

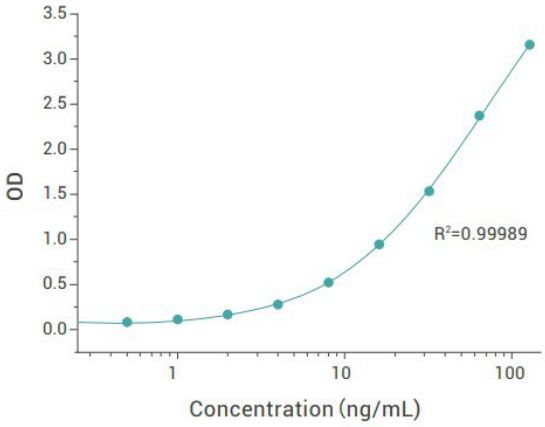
5. Wash: Remove the cover and discard the plate content, and wash plate 3 times with 1× PBST Wash Buffer (300µl/well). Pat dry on filter paper after each wash. Do NOT let the wells dry completely at any time.
6. Add 100ul of Streptavidin HRP Working Solution into each well, cover the plate and incubate at 37°C for 60 minutes. (Note: Orbital shaking at 200-300 rpm is recommended for the duration of the incubation.)
7. Wash: Remove the cover and discard the plate content, and wash plate 3 times with 1× PBST Wash Buffer (300µl/well). Pat dry on filter paper after each wash. Do NOT let the wells dry completely at any time.
8. TMB Substrate: Add 100ul TMB Substrate into each well, cover the plate and incubate at 25°C in dark for 10 minutes.
9. Add 50ul Stop Solution into each well and mix slightly. The color will turn yellow immediately. The adding order of Stop Solution should be as the same as the TMB Substrate Solution.
10. Select the main wavelength of the microplate 450 nm and the reference wavelength 630 nm. Read the O.D. absorbance in Microplate Reader immediately after adding the stop solution.

Typical Standard Curve

It is recommended to use the 4-Parameter fitting method for linear fitting and calculation of this product.

Results of a typical standard operation of a DNase I ELISA Kit are listed below. This standard curve was generated at our lab for demonstration purpose only. Users shall obtain standard curve as per experiment by themselves.

STD (ng/mL)	OD-1	OD-2	Average
64	2.373	2.347	2.360
32	1.520	1.546	1.533
16	0.933	0.934	0.9335
8	0.518	0.536	0.527
4	0.259	0.291	0.275
2	0.166	0.168	0.167
1	0.115	0.109	0.112
0	0.057	0.049	0.053



Precision

CV%<10%, RE%<±15%

Detection Range

1-64ng/mL

Sensitivity

0.5ng/mL

Precautions

1. If the test samples are purified products, it is usually recommended to test a stock solution or a 2-fold dilution. At least three consecutive fold dilutions were recommended for the first test to produce at least one diluted sample within the range of the standard curve. Before further analysis or dilution, the diluent shall be fully mixed. Repeat the analysis twice for each sample to determine the correct DNase I residue value in the original sample.
2. Reagents should be stored according to label instructions and equilibrated at room temperature before use.
3. Balance microplate to room temperature before opening the outer packaging bag. The slats not used in the experiment should be put back into the packaging immediately and sealed, which can be stored for 1 month at 4°C. The remaining reagents should be packed or covered.
4. Before using the kit, spin tubes and bring down all components to the bottom of tubes.
5. Don't reuse tips and tubes to avoid cross contamination.
6. Check reagents in the kit before use. It is particularly important for the experimental results that reagent dilution, sample addition and reaction termination should be fully mixed or shaken.
7. The washing buffer remaining in the reaction wells should be thoroughly pat dry on clean filter paper until there is no watermark. Do not put the filter paper directly into the reaction wells to absorb water.
8. Storage TMB reagents avoid light. Avoid contact with metals to affect the results.
9. This kit is disposable, please use it within the validity period.