Instructions for Use of DNase I ELISA Detection Kit

The kit is intended for scientific research only and should not be used for diagnosis

Cat. No. HG- DI001

Introduction

This detection kit is applicable for the quantitative detection of residual DNase I content in test samples by double-antibody sandwich method. The standard and test samples are added to reaction wells on microplate pre-coated with DNase I-specific monoclonal antibodies for incubation. The present DNase I quantitatively binds to the antibodies in the microplate, while unbound substances are removed after plate washing. Add Anti-DNase I mAb (detection antibody) and H + L secondary antibodies successively (enzyme conjugate) to form an antibody - antigen - antibody - secondary antibody complex. The protein content in the test sample can be indicated by the color development degree of TMB. Please read the instructions for use carefully and check the components of the kit before use.

Assay range: 1-64 ng/mL
Limit of quantification: 1 ng/mL
Limit of detection: 0.5 ng/mL
Precision: CV%≤10%, RE%≤±15%

Specification

96 T.

Usage

This kit is suitable for rapid detection of DNase I content in test samples.

Kit components

Components	Specification	Preparation
DNase Ⅰ Coated Plate	8 wells × 12 strips	Ready-to-use
Anti-DNase I (detection antibody)	150 μL × 1 vial	1:100, dilute with Antibody Diluent Buffer
Streptavidin HRP (enzyme conjugate)	150 μL × 1 vial	1:100, dilute with Enzyme Conjugate Diluent Buffer
DNase I Standard (standard)	30 μL × 1 vial (100 μg/mL)	Operate as per the recommended dilution procedure
Sample Diluent Buffer	60 mL× 1 bottle	Ready-to-use
Antibody Diluent Buffer	12 mL× 1 bottle	Ready-to-use
Enzyme Conjugate Diluent Buffer	12 mL× 1 bottle	Ready-to-use
20×PBST Wash Buffer	50 mL× 1 bottle	1: 20, dilute with deionized water
TMB Substrate	11 mL× 1 bottle	Ready-to-use
Stop Solution	7 mL× 1 bottle	Ready-to-use
Plate Sealer	5 pieces	Ready-to-use
Instructions for Use	1 copy	/

Notes: Detection antibody, enzyme conjugate, and standard should be stored at -18℃, and other components should be

stored at 2 ~ 8℃ away from light. The shelf life is 12 months.

Apparatus and materials to be prepared by the user:

(1) Plate reader
(2) Thermostat plate shaker
(3) Micro pipette and tips
(4) Deionized water
(5) Unused filter paper
(6) Vortex shaker

Reagent preparation

(1) Temperature equilibration: Transfer reagents to be used to room temperature (18 ~ 25℃) environment and equilibrate the temperature for 30 minutes.
(2) Preparation:
① 1× PBST Wash Buffer: Calculate the volume of working buffer required, measure an appropriate amount of 20× PBST Wash Buffer, dilute with deionized water at 1:20, and mix well for later use.
② Detection antibody working buffer: Calculate the volume of working buffer required, measure an appropriate amount of antibody, dilute with Antibody Diluent Buffer at 1:100, and mix well for later use.
③ Enzyme conjugate working buffer: Calculate the volume of working buffer required, measure an appropriate amount of enzyme conjugate, dilute with Enzyme Conjugate Diluent Buffer at 1:100, and mix well for later use.
④ The standard and test samples should be diluted with the Diluent Buffer.
(3) Dilution of standard

Vial No.	Standard solution concentration(ng/mL)	Standard solution volume (μL)	Diluent Buffer volume (μL)	Total volume (μL)	Final concentration (ng/mL)	Remaining volume (μL)
Pre-1	100000	5	45	50	10000	42
Pre-2	10000	8	617	625	128	325
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

Operation procedures

(1) Mix all reagents well before use to avoid bubbles.

(2) Confirm the number of stripe plates required based on the number of experimental wells. Put remaining strip plates back to aluminum foil bags with desiccants and seal the bag.

(3) Loading: Add standard, sample dilution working buffer, and negative control into respective wells at 100 μL/well. After sealing the plate with a plate sealer, place the plate in a thermostat shaking incubator at 37℃, and incubate for 60 minutes at 200 ~ 300 rpm.

(4) Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 μL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on the filter paper after each washing.

(5) Addition of detection antibody working buffer: Add 100 μL of detection antibody working buffer to each well. After

sealing the plate with a plate sealer, place the plate in a thermostat shaking incubator at 37℃, and incubate for 60 minutes at 200 ~ 300 rpm.

(6) Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.

(7) Addition of enzyme conjugate working buffer: Add 100 µL of enzyme conjugate working buffer to each well. After sealing the plate with a plate sealer, place the plate in a thermostat shaking incubator at 37℃, and incubate for 60 minutes at 200 ~ 300 rpm.

(8) Plate washing: Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.

(9) Color development: Add 100 µL of TMB Substrate to each well, gently shake to mix well, seal the plate with a plate sealer, and place the plate at 25℃ for 10 minutes for color development reaction.

(10) Assay: Add 50 µL of Stop Solution to each well and gently shake to mix well. Measure the optical density (OD) value of each well with a microplate reader at a primary wavelength of 450 nm and a reference wavelength of 630 nm.

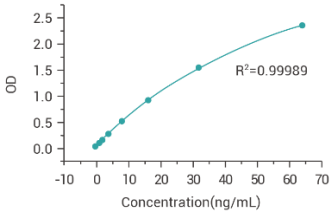
Results process

The 4-parameter fitting method is recommended for the linear fitting and calculation of the product.

(1) OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail)

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
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

(2) The standard curve is obtained by 4-parameter fitting with the theoretical standard concentrations and the corresponding OD values (as shown in the figure below)



Limitations of the assay method

The reagent is for assay of DNase I in test samples only.

Precautions

(1) If the test samples are purified, it is usually recommended to detect with the original solution or 2-fold diluted solution. When testing for the first time, it is recommended to perform dilution with at least 3 consecutive dilution factors, so as to generate at least one diluted sample within the range of the standard curve. Diluent should be mixed thoroughly before further analysis or dilution. Each sample should be analyzed in duplicate to determine the correct DNase I residue in the original sample.

(2) The reagents should be stored as indicated on the label, and should be equilibrated to room temperature before use.

(3) Before using the pre-coated strip plates, please equilibrate to room temperature before opening the secondary packaging. Strip plates not used in the experiment should be put back to the package immediately, and the package should be sealed tight. The plates may be stored for 1 month at 4℃. Other unused reagents should be packaged or covered.

(4) The volumes of standard, biotin, and enzyme conjugate are all very small. Please perform rapid centrifugation before use to let liquid on the tube wall or cap gather at tube bottom.

(5) Please use disposable tips during experimental operation to avoid cross contamination.

(6) Please check each reagent in the kit before use. To obtain accurate assay results, it is of special importance to mix well or shake well the reagents for dilution, loading, and reaction termination.

(7) When washing residual Wash Buffer in the reaction wells, pat the plate dry adequately on clean tissue papers until watermark is no longer visible. Do not put the tissue paper into the well to absorb the liquid.

(8) The TMB Substrate is photosensitive, thus long-time exposure to illumination should be avoided; avoid contact with metal, otherwise, the assay results may be affected.

(9) The kit is for single use. Please use within the shelf life.

Disclaimer

Under all circumstances, the liability of our company for this product is only limited to the value of the product itself.

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