Version 22-04/24

User's Manual

DNase I ELISA Kit

REF **DEIANS026**

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.

Creative Diagnostics

Address: 45-1 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-624-4882 (USA) 44-161-818-6441 (Europe) **Fax: 1-631-938-8221**

Email: info@creative-diagnostics.com **Web: www.creative-diagnostics.com**

PRODUCT INFORMATION

Intended Use

This kit employs double-antibody sandwich enzyme-linked immunosorbent assay to detect the content of DNase I.

General Description

Deoxyribonuclease I (usually called DNase I), is an endonuclease of the DNase family coded by the human gene DNASE1. DNase I is a nuclease that cleaves DNA preferentially at phosphodiester linkages adjacent to a pyrimidine nucleotide, yielding 5'-phosphate-terminated polynucleotides with a free hydroxyl group on position 3', on average producing tetranucleotides. It acts on single-stranded DNA, double-stranded DNA, and chromatin. In addition to its role as a waste-management endonuclease, it has been suggested to be one of the deoxyribonucleases responsible for DNA fragmentation during apoptosis.

DNase I binds to the cytoskeletal protein actin. It binds actin monomers with very high (sub-nanomolar) affinity and actin polymers with lower affinity. The function of this interaction is unclear. However, since actin-bound DNase I is enzymatically inactive, the DNase-actin complex might be a storage form of DNase I that prevents damage of the genetic information. This protein is stored in the zymogen granules of the nuclear envelope and functions by cleaving DNA in an endonucleolytic manner.

Principles of Testing

This kit employs double-antibody sandwich enzyme-linked immunosorbent assay (sandwich ELISA) to detect the content of DNase I. Coat the microplate with anti-DNase I monoclonal antibodies to form solid-phase antibodies, add the DNase I standard and the sample to be tested into the solid-phase antibody microplate, then add horseradish peroxidase (HRP)-labeled anti-DNase I monoclonal antibodies after washing to form "coating antibody-antigen-enzyme-labeled detection antibody" complexes. Add the TMB substrate solution after washing to develop color (the TMB substrate solution turns blue under the catalysis of HRP enzyme, and the color finally changes to yellow with an acid. The color intensity is positively correlated with the amount of DNase I in the sample).

Reagents And Materials Provided

Box 1

1. DNase I standard (8,000 ng/ml), 100 µl

Box 2

1. Pre-coated microplate (coated with anti-DNase I monoclonal antibody), 12×8 wells, 96 wells
2. Sample diluent, 30 ml
3. Enzyme-labeled reagent diluent, 12 ml
4. Enzyme-labeled reagent (100×), 120 µl
5. Concentrated wash buffer (20×), 30 ml
6. TMB substrate solution, 12 ml

7. Stop solution, 6 ml
8. Sealing film, 3 pcs
9. Instructions for Use, 1 pcs

Note: The components in this kit shall not be used interchangeably with those in other commercially available kits or those from different lots.

Materials Required But Not Supplied

1. Deionized or distilled water
2. Shaker
3. Plate washer
4. Micropipettes and compatible sterile tips
5. Thermostatic incubator or water bath
6. Microplate reader
7. Sample loading slot
8. Absorbent pad

Storage

1. For Box 1, store at -30 to -15°C and transport at ≤ 0°C. For Box 2, store at 2-8°C. Protect the kit from light, and do not freeze it.
2. See the label for the production date and expiration date. The shelf life is 12 months.

Specimen Collection And Preparation

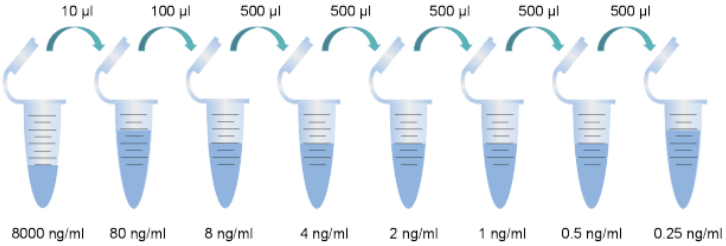
Dilute the sample to be tested with the sample diluent by a factor of at least 5 before testing.

Reagent Preparation

Note: Take out the kit from the refrigerator and the standard from the freezer, and equilibrate them at room temperature (18-28°C) for at least 30 minutes.

1. Preparation of wash buffer (1×): Dilute the concentrated wash buffer (20×) with deionized water or distilled water by a factor of 20, and mix well for later use. For example, dilute 30 ml of concentrated wash buffer (20×) with 570 ml of deionized or distilled water.

2. Preparation of standard: Dilute the stock solution of the standard (8,000 ng/ml) by a factor of 100 to 80 ng/ml followed by a factor of 10 as the first concentration point of the calibration curve (8 ng/ml), and then continue serial dilution by a factor of 2 to 4 ng/ml, 2 ng/ml, 1 ng/ml, 0.5 ng/ml, and 0.25 ng/ml. To ensure the validity of the test results, please use a freshly prepared standard solution for each test. **Note: Aliquot and store the standard after initial use.**



Pipette	Into	Concentration of Prepared DNase I Standard
10 µl of 8,000 ng/ml standard	990 µl of sample diluent	80 ng/ml
100 µl of 80 ng/ml standard	900 µl of sample diluent	8 ng/ml
500 µl of 8 ng/ml standard	500 µl of sample diluent	4 ng/ml
500 µl of 4 ng/ml standard	500 µl of sample diluent	2 ng/ml
500 µl of 2 ng/ml standard	500 µl of sample diluent	1 ng/ml
500 µl of 1 ng/ml standard	500 µl of sample diluent	0.5 ng/ml
500 µl of 0.5 ng/ml standard	500 µl of sample diluent	0.25 ng/ml
500 µl of sample diluent	Empty tube (control well)	0 ng/ml

3. Preparation of enzyme-labeled reagent (1×): Use the enzyme-labeled reagent diluent to dilute the enzyme-labeled reagent (100×) to the enzyme-labeled reagent (1×). Determine the dilution volume (100 µl diluted reagent per well) according to the number of tests. For example, to test the whole plate, 10 ml of enzyme-labeled reagent (1×) is theoretically required, while 11 ml of enzyme-labeled reagent (1×) is actually prepared. In other cases, prepare 10% more of the theoretical volume required for the specific number of tests. Mix the solution well by shaking it upside down at least 30 times to prepare the enzyme-labeled reagent (1×).

Assay Procedure

1. Take out the kit from the refrigerator and the standard from the freezer, and equilibrate them at room temperature (18-28°C) for at least 30 minutes.
2. Take out a required number of pre-coated microplate strips, keep the remainder in a ziplock bag and put it back at 2-8°C.
3. **Sample loading:** Add the sample and standard at 100 µl per well into the pre-coated microplate.
4. **Incubation:** Seal the plate with a sealing film and incubate it in a 37°C thermostatic incubator for 1 hour.
5. **Plate washing:** Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of wash buffer (1×) into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
6. **Addition of enzyme-labeled reagent:** Add the enzyme-labeled reagent (1×) at 100 µl per well.
7. **Incubation:** Seal the plate with a sealing film and incubate it in a 37°C thermostatic incubator for 1 hour.
8. **Plate washing:** Upon the completion of incubation, carefully remove the sealing film, and discard the liquid in the wells. Add at least 300 µl of wash buffer (1×) into each well, and leave it to soak for 30 seconds. Wash the plate 4 times, and remove all of the residual liquid to the extent possible in the last washing.
9. **Color development:** Add the TMB substrate solution into the plate at 100 µl per well, seal the plate with a

sealing film, and incubate it in a 37°C thermostatic incubator for 15 minutes (keep away from light).

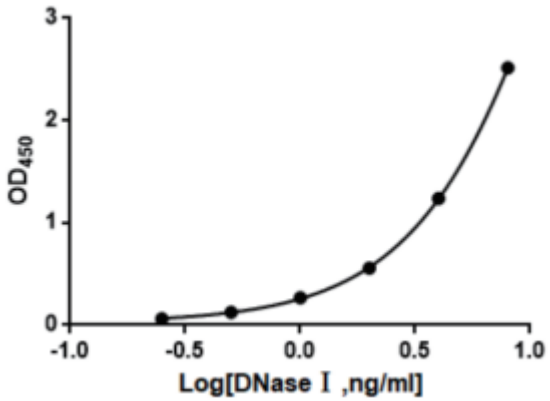
10. **Termination/Reading:** Add the stop solution at 50 µl per well, gently mix well and then use a microplate reader to determine the absorbance per well at a single wavelength of 450 nm within 10 minutes.

Calculation

1. Subtract the signal value of the control well from the measured signal value of the standard well. Plot the calibration curve by four-parameter fitting with the logarithm of the concentration of the standard as the x-coordinate and the OD value as the y-coordinate. If replicate wells are set, the calculation should be based on the mean value.
2. Substitute the measured OD value of the sample well minus the signal value of the control well into the fitting equation of the calibration curve and calculate the sample concentration, i.e., the actual concentration of the sample. The limit of quantitation (LOQ) = 250 pg/ml. Values below 250 pg/ml should be reported as < 250 pg/ml. If the sample's OD value is above the upper limit of the calibration curve, a retest should be performed after appropriate dilution, and the concentration should be calculated by multiplying the dilution factor. The calibration curve below is for demonstration purposes only, and a new calibration curve should be generated for each test.

Typical Standard Curve

DNase I Concentration (ng/ml)	OD_{450}-$OD_{control\ well}$
8	2.5170
4	1.2396
2	0.5587
1	0.2693
0.5	0.1264
0.25	0.0626
0	0.0000
R^2	0.9999



Precision

	Within-Run Precision			Between-Run Precision		
Sample	1	2	3	1	2	3
n	10	10	10	30	30	30
Mean	4.18	0.91	0.29	4.11	0.89	0.28
SD	0.13	0.01	0.01	0.16	0.03	0.01
CV	3%	1%	3%	4%	3%	4%

Sensitivity

LOD	LOQ
<50 pg/ml	250 pg/ml

Recovery

Sample (n = 10)	Mean Measured Concentration (ng/ml)	Mean Recovery (%)	Recovery Range (%)
4 ng/ml	3.99	100	95 - 108
1 ng/ml	0.87	87	83 - 92
0.25 ng/ml	0.28	111	105 - 118

Precautions

1. For research use only. Not for use in diagnostic procedures.The pre-coated microplate is removable. After taking off the required number of plate strips each time, keep the remainder in an aluminum foil pouch and store it at 2-8°C for later use. Do not touch the bottom of the well when detaching the required strips from the plate to avoid fingerprints or scratches that may affect subsequent readings. After plate washing, immediately perform the next operation; otherwise, the plate may get dry.
2. Do not reuse the sealing film.
3. Store and use each component in strict accordance with the Instructions for Use, and do not change or dilute the component arbitrarily.
4. Carefully check the expiration date and packaging of the kit before use. If the kit expires or its package is damaged, do not use it for tests.
5. All reagents should be equilibrated to room temperature before preparation and use, and then immediately put back at 2-8°C after use, while the standard should be put back at -30 to -15°C.
6. When loading the sample, avoid bubbles, and prevent the pipette tip from touching the bottom of the plate, which may cause scratches and affect the readings.
7. Wear disposable gloves and protective gear in accordance with laboratory regulations during the operation. After the test, dispose of the liquid waste and disposable consumables in a harmless way in accordance with relevant local and national regulations.