BIORI BIOTECH

Principle

This product uses the ELISA double-antibody sandwich method to determine the content of DNase I in samples. Anti-DNase I antibodies are coated on microtiter strips. Standards or test samples and Anti-DNase I detection antibodies are added, and followed by incubation and washing. A substrate solution (3,3',5,5'-tetramethylbenzidine, TMB) is added for color reaction. TMB is colorless initially and turns blue under the catalysis of HRP, and finally turns yellow under acidic conditions. The intensity of the yellow color is positively correlated with the amount of DNase I in the sample.

Specification

96 T/ Kit

Usage

This kit is used for the quantification of DNase I in samples.
This kit is research and manufacture use only and is not intended for medical diagnosis.

Product Performance

This kit has been fully evaluated, with a standard curve range of 1-64 ng/ml and a quantitation limit of 0.1 ng/mL. The intra-batch CV is less than 10%, and inter-batch CV is less than 15%; the recovery rates are between 80% and 120%.

Main Components

	Components	Specification	Storage temperature
Box 1	DNase I standard (25.6 μg/mL)	30 μL	-20±5°C
Box 2	Anti-DNase I detection antibody	12 mL	2-8°C
	Concentrated Wash Buffer (20×)	25 mL	
	Sample Diluent	25 mL	
	TMB Substrate	12 mL	
	Stop Solution	6 mL	
	Anti-DNase I coated microplate	12*8 wells strips	
	Sealing film	2 sheet	
	One Instruction Manual	/	

Items that need to be prepared additionally

1. Vortex Oscillator
2. Microplate washer
3. Constant Temperature Micro-plate Shaker
4. Micro-plate Reader
5. Deionized water
6. Pipettor and tips
7. Absorbent paper,etc.

Storage conditions and shelf life

1. The shelf life of an unopened product is 12 months. After opening, the product is valid for 1 month (within the expiration date).

2. After the micro-plate is unsealed and used, the remaining portion should be stored sealed in an aluminum foil bag.

3. After use, the kit should be promptly returned and stored under its specified storage conditions.

4. The product batch number and expiration date can be found on the product packaging label.

Precautions

1. This kit is only used for research.

2. All samples and reagents are considered potentially hazardous. For your safety and health, please wear protective measures such as lab coats and disposable gloves. Avoid direct contact of reagents with skin and eyes. In case of accidental contact, rinse immediately with plenty of water.

3. Please read the product manual carefully before using this kit.

4. Please use the product within the expiration date indicated on the kit, and store each component according to the label conditions. Components from different batch numbers should not be mixed. This kit should not be used with other commercial kits.

5. The standard is supplied in a small volume. Before use, centrifuge at high speed to collect liquid from the tube wall and cap to the bottom.

6. To avoid microbial contamination, as well as cross-contamination between reagents and samples, please use disposable containers. The sealing film is disposable.

7. It is recommended to use a plate washer for washing plates. During the washing process, avoid cross-flow or bubbles to prevent affecting the accuracy of the experimental results. After the final wash, any remaining liquid in the wells should be emptied.

8. Result measurement should be performed within 15 minutes after stopping the reaction.

9. A new standard curve needs to be generated for each measurement.

10. For your safety and health, please wear laboratory coats and disposable gloves.

11. This kit is for research use only.

Detection method

1. Temperature equilibration

Bring all reagents to room temperature (25±2°C) for at least 30 minutes.

2. Reagent Preparation

Salt precipitate may be observed in concentrated buffer, please place it in a 37°C water bath until complete dissolution.

2.1 Washing solution

After allowing the concentrated wash solution (20×) to reach room temperature (25±2°C), gently mix it. Calculate the required amount of wash solution according to the experiment. Dilute and prepare it at a volume ratio of concentrated wash solution (20×) to purified water = 1:19, gently mix to avoid excessive foaming. Prepare fresh as needed.

2.2 Standard working solutions

Prepare DNase I working solutions as the following table:

No.	Higher-level standard (ng/mL)	Standard need (μL)	Sample dilute volume (μL)	Total volume (μL)	Final concentration (ng/mL)	Remain Volume (μL)
A	25600	2	798	800	64	500
B	64	300	300	600	32	300
C	32	300	300	600	16	450
D	16	150	450	600	4	300
E	4	300	300	600	2	300
F	2	300	300	600	1	600
G	/	0	300	300	0	300

3. Sample dilution

It is recommended to conduct spike recovery studies on the samples to eliminate the interference of the sample matrix and ensure the accuracy and reliability of Anti-DNase I detection results. It is recommended to dilute the samples using the minimum dilution factor.

If your sample is a process stage sample and its concentration is higher than the analytical range of this detection method, it can be diluted at a higher than minimum dilution factor, but the dilution linearity should be assessed to ensure that the detection method is accurate and has sufficient antibody excess to be suitable for your HCP.

4．Assay Protocol

4.1 Micro-plate well

Take out the corresponding number of strips from the aluminum foil bag according to the experiment need.

4.2 Add detection antibody

Pipette 100 μL of detection antibody to each well

4.3 Add standards/samples

Pipette 100 μL of DNase I standards of various concentrations, the prepared samples, and the negative control. Be sure to add the standards, samples, controls, and detection antibodies continuously within 15 minutes. Seal the plate with a plate seal, and incubate at room temperature (25±2°C) on a micro-plate shaker for 60 minutes, keeping the shaker speed at 350 rpm (the speed can be adjusted according to different shakers to ensure the solution in each well is thoroughly mixed without splashing).

4.4 Washing

Discard the liquid in each well, then add 350 μL of washing solution to each well. After standing for 30 seconds, discard the liquid in the wells; repeat this procedure 4 times. After the final wash, dry the plate by patting it on blotting paper.

4.5 Add Substrate

Pipette 100 μL of substrate solution to each well, keep at room temperature (25±2°C) and incubate in the dark for 20 minutes.

4.6 Stop the reaction

Pipette 50 μLof stop solution to each well, mix gently, and observe the color change from blue to yellow.

4.7 Signal detection

Select the micro-plate reader's main wavelength at 450 nm and the reference wavelength at 630 nm to measure the absorbance values of each well at 450 nm and 630 nm. Please read the data within 15 minutes after the reaction is stopped.

5．Result Analysis

5.1 The OD value of each well = absorbance at 450 nm - absorbance at 630 nm. If the micro-plate reader is not equipped with a reference wavelength (630 nm), the OD value of each well = absorbance at 450 nm. If the OD value of the sample to be tested exceeds the maximum value of the standard curve, the sample should be further diluted before testing.

5.2 It is recommended to use the concentration of the standard as the x-axis and the OD value as the y-axis for this kit, and fit the curve using a four-parameter method. The standard curve fitting can be done using the software that comes with the micro-plate reader. If that is not available, it is recommended to use professional standard curve software, such as Curve Expert or ELISA Calc. The correlation coefficient R^2 of the standard curve should be ≥0.99; otherwise, the experiment is invalid.

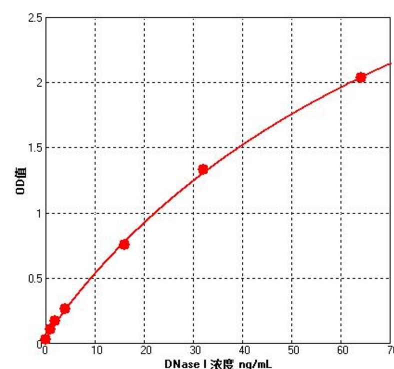
5.3 It is recommended to set up a repeat hole, and the average value should be calculated before performing curve fitting.

5.4 Substitute the OD value of the sample to be tested into the fitted equation of the standard curve to calculate the sample concentration, which represents the concentration of Anti-DNase I in the sample. If the sample to be tested has been diluted, the calculated result should be multiplied by the dilution factor.

Typical Standard Curve

A typical standard curve is shown below; the data is for reference only and the actual test results shall prevail.

DNase I ng/mL	OD value
64	2.037
32	1.330
16	0.758
4	0.266
2	0.174
1	0.111
0	0.032



Zhuhai Biori Biotechnology Co.,Ltd
Tel: +86-0756-8699969 Web: www.biori-en.com Email: marketing@biori-en.com
Addr:No. 88, Shui’ an 1st Road, Xiangzhou District, Zhuhai, Guangdong 519060, China

Common Issue Troubleshooting

Abnormal phenomenon	Possible cause	Solution approach
The overall OD values of the experiment are relatively low	The kit is not fully balanced	Take the kit out of the refrigerator before the experiment and let it equilibrate at 25°C for at least 30 minutes before use.
	Improper mixing or storage of reagents	Reagents from different batches should not be mixed; please store each reagent according to the instructions in the manual.
	Incubation time/temperature does not match the instructions	Please strictly follow the instructions for incubation. In winter, when the room temperature is low, if the color development is lighter, you can appropriately extend the color development time or ensure the room temperature is adjusted to around 25°C.
	Improper sample preparation	Re-prepare standard curves and samples
The overall OD values of the experiment are higher than normal	Incubation time/temperature does not match the instructions	Please strictly follow the instructions in the manual for the incubation reaction.
	Improper sample preparation	Re-prepare standard curves and samples
Standard curve is normal, but the sample shows abnormal coloration	The sample dilution factor is not appropriate	It is recommended to make 4-5 dilution gradients for the initial test samples to ensure that at least one value falls within the detection range.
	Hook effect	Increase the dilution factor while ensuring the concentration remains within the detectable range.
	Improper sample storage or too many freeze-thaw cycles	Try to use aliquoted samples that have been frozen for no more than 3 months; use aliquots to minimize repeated freeze-thaw cycles.
	There are interfering factors in the sample solution	While ensuring that the detected concentration falls within the detection range, increase the dilution factor to reduce interference; if the issue cannot be resolved, contact our technical staff for specialized adjustments.
	The content of the analyte in the sample is too low	Reduce the dilution factor or concentrate the sample before adding it
High background value	Incubation time/temperature does not match the instructions	Please strictly follow the instructions in the manual for the incubation reaction.
	The micro-plate was not covered with a sealing film before the reaction	During incubation, cover with a sealing film to prevent liquid evaporation and contamination.
	Abnormalities in the washing or sample-loading process may inadvertently introduce contamination	Use the washing solution provided with the kit, ensure the number of washes and the volume of the washing solution are sufficient, and make sure the wells are clean and free of residue after washing; ensure the laboratory work area is clean.
	Color shows without light protection / long duration	Color development should be done away from light, and the color development time should refer to the

		instructions.
The coefficient of variation (CV) is relatively high	The pipette is inaccurate	Use a calibrated pipette and operate it correctly
	Edge effect	Ensure that all reagents and micro-plates are at the same temperature
	Reagent mixing	Reagents from different batches should not be mixed.
Spike recovery exceeds the acceptable range	Matrix interference	Assess matrix effects through linear dilution and recovery experiments. Increase the dilution factor to reduce interference while ensuring the detected concentration falls within the detectable range; if the issue cannot be resolved, contact our technical staff for specialized adjustments.
	The selected **spiking concentration is inappropriate**	The spiked concentration is best set higher than the concentration of the target analyte in the actual sample; when selecting the spiked concentration at low levels, it should not be lower than the quantitation limit.