EasyAna UniversalBenzo Nuclease Quantitative Detection Kit (ELISA)

DD3501EN

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Instruction for Use

Version 22.1

Vazyme

Contents

01/Intended Use

This kit employs double-antibody sandwich enzyme-linked immunosorbent assay (sandwich ELISA) to detect the residual UniversalBenzo Nuclease.

02/Test Principle

UniversalBenzo Nuclease is a non-specific broad-spectrum endonuclease from *Serratia marcescens* and has been genetically modified. It can degrade double-stranded, single-stranded, circular, or linear DNAs and RNAs and fully digest nucleic acids into 5'-monophosphate oligonucleotides with a length of 2 - 5 bases. The enzyme can efficiently degrade all forms of RNAs and DNAs under a wide range of conditions, so it is widely used to remove the residual nucleic acids and contaminants in biological products.

This kit employs sandwich ELISA to determine the content of UniversalBenzo Nuclease. Coat the microplate with anti-UniversalBenzo Nuclease monoclonal antibodies to form solid-phase antibodies, add the UniversalBenzo Nuclease standard and the test sample to the solid-phase antibody microplate, then add horseradish peroxidase (HRP)-labeled anti-UniversalBenzo Nuclease monoclonal antibodies to form "coating antibody-antigen-enzyme-labeled reagent" complexes. Add the TMB substrate solution after washing to develop color (the TMB substrate solution turns blue under the catalysis of HRP, and the color finally changes to yellow with an acid). The color intensity is positively correlated with the level of UniversalBenzo Nuclease in the sample.

03/Key Components

Component	DD3501EN-01
1. Pre-coated microplate (coated with anti-UniversalBenzo Nuclease monoclonal antibody)	12 × 8 wells, 96 wells
2. UniversalBenzo Nuclease standard (800 ng/ml)	0.1 ml
3. Sample diluent	30 ml
4. Enzyme-labeled reagent diluent	12 ml
5. Enzyme-labeled reagent (1,000×)	50 µl
6. Concentrated wash buffer (20×)	30 ml
7. TMB substrate solution	12 ml
8. Stop solution	6 ml
9. Sealing film	3 pcs
10. 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.

Reagents and instruments required but not provided:

>Deionized water
>Sample loading slot
>Liquid waste tank
>Microplate reader
>Microplate mixer
>Plate washer
>Thermostatic incubator
>Timer
>Pipettes and compatible sterile tips
>Disposable gloves
>Absorbent pad

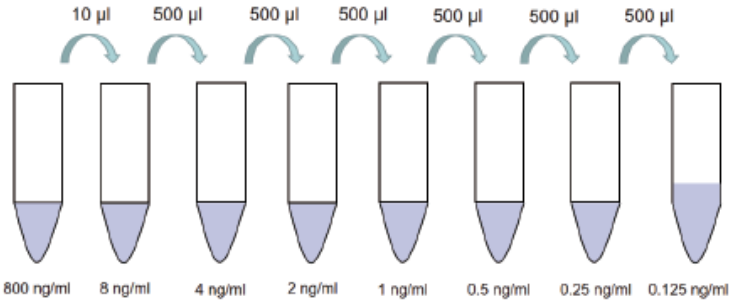
04/Storage and Shelf Life

1. Store the kit at 2 ~ 8℃, avoid freezing and protect it from direct bright light. The kit has a shelf life of 12 months.
2. See the label for the production date and expiration date.

05/Test Method

05-1/Test Preparation

1. Take out the kit from the refrigerator and equilibrate it at room temperature (18 ~ 28℃) for at least 30 minutes.
2. 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.
3. Take out a required number of pre-coated microplate strips, keep the remainder in a ziplock bag and put it back at 2 ~ 8℃.
4. Preparation of standard: Dilute the stock solution of the standard (800 ng/ml) by a factor of 100 to 8 ng/ml as the first concentration point, and then continue serial dilution by a factor of 2 to 4 ng/ml, 2 ng/ml, 1 ng/ml, 0.5 ng/ml, 0.25 ng/ml, and 0.125 ng/ml. To ensure the validity of the test results, please use a freshly prepared standard solution for each test.



Pipette	Into	Concentration of Prepared UniversalBenzo Nuclease
10 µl of 800 ng/ml standard	990 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 0.25 ng/ml standard	500 µl of sample diluent	0.125 ng/ml
500 µl of sample diluent	Empty tube	0 ng/ml

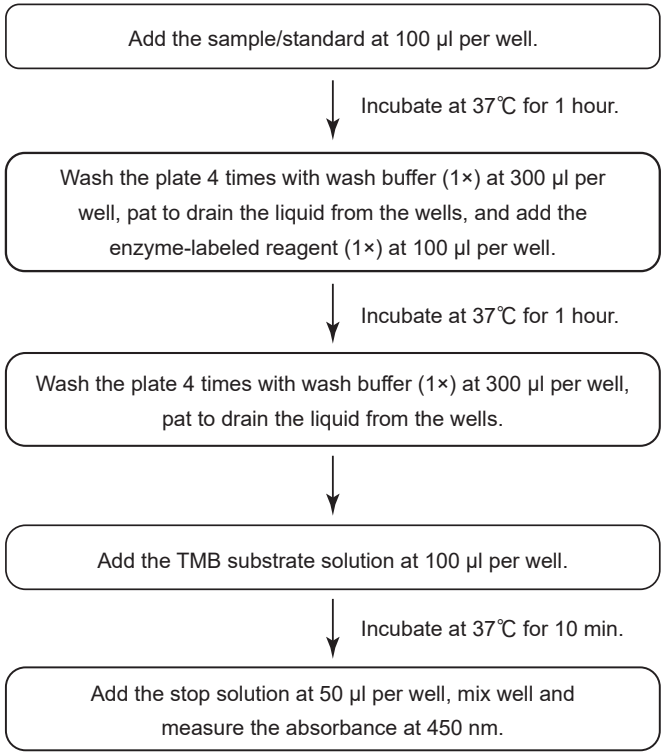
5. Preparation of enzyme-labeled reagent (1×): Add 10 µl of the enzyme-labeled reagent (1,000×) to 9.99 ml of the enzyme-labeled reagent diluent. Mix the solution well by shaking it upside down at least 30 times to prepare the enzyme-labeled reagent working solution (1×).

05-2/Test Operation

1. Sample loading: Add the sample/standard at 100 µl per well to the pre-coated microplate.
2. Incubation: Seal the plate with a sealing film and incubate it in a 37℃ thermostatic incubator for 1 hour.
3. 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.
4. Addition of enzyme-labeled reagent working solution: Add the enzyme-labeled reagent working solution (1×) at 100 µl per well.
5. Incubation: Seal the plate with a sealing film and incubate it in a 37℃ thermostatic incubator for 1 hour.

7. Color development: Add the TMB substrate solution to the plate at 100 µl per well, seal the plate with a sealing film, and incubate it in a 37℃ thermostatic incubator for 10 minutes.
8. Termination/Reading: Add the stop solution at 50 µl per well and gently mix well. Read the value within 10 minutes. Measure the single-wavelength absorbance at 450 nm per well with a microplate reader.

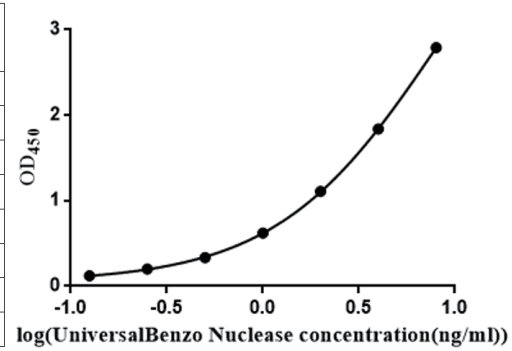
06/Brief Operating Procedure



07/Result Interpretation

1. Plot the standard 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. Calculate the sample concentration, i.e., the actual concentration of the sample. The limit of quantitation (LOQ) = 125 pg/ml. Values below 125 pg/ml should be reported as < 125 pg/ml. If the sample's OD value is above the upper limit of the standard curve, a retest should be performed after appropriate dilution, and the concentration should be calculated by multiplying the dilution factor. The standard curve below is for demonstration purposes only, and a new standard curve should be generated for each test.

Concentration (ng/ml)	OD450 Value
8	2.7916
4	1.8395
2	1.1085
1	0.6205
0.5	0.3342
0.25	0.2002
0.125	0.1221
0	0.0477



08/Performance Indicators

08-1/Sensitivity

LOD	LOQ
18.6 pg/ml	125 pg/ml

08-2/Precision

	Within-Run Precision			Between-Run Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean of OD	3.91	0.97	0.47	3.79	1.05	0.50
SD	0.29	0.05	0.03	0.10	0.07	0.02
CV	7%	5%	6%	3%	7%	4%

08-3/Recovery

Sample (n=3)	Measured Concentration (ng/ml)	Mean Measured Concentration (ng/ml)	Mean Recovery (%)	Recovery Range (%)
4 ng/ml	4.76 4.43 4.16	4.45	111	104 - 119
1 ng/ml	1.02 1.10 1.07	1.06	106	102 - 110
0.25 ng/ml	0.25 0.24 0.26	0.25	100	97 - 104

09/Notes

For research use only. Not for use in diagnostic procedures.

1. 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℃ 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 re-use 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℃ after use.
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.