Instructions for Use of Benzonase Nuclease ELISA Detection Kit

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

Cat. No. HG- BE001

Introduction

The Benzonase Nuclease ELISA Detection Kit is designed for the quantitative detection of residual nuclease content in intermediates, semi-finished products and finished products of various biological products.

The kit can quantitative detect trace residual Benzonase nuclease (hereinafter referred to as "nuclease") in test samples by using a double-antibody sandwich method. Coat the 96-well plate with capture antibody to prepare immobilized antibodies. Add the standard and test samples, and then horseradish peroxidase (HRP) conjugated antibody to form an immobilized antibody - nuclease - conjugated antibody sandwich conjugate. Wash the plate after reaction, and add the substrate for color development reaction. The substrate will turn blue under HRP catalysis, and will finally turn yellow under the action of the stop solution. Determine the optical density (OD) value at 450 nm, and calculate the nuclease content in the test sample using the standard curve.

Specification

96 T

Kit components

Components	Specification	Preparation
Benzonase Coated Plate	8 wells × 12 strips	Ready-to-use
Benzonase Standard (standard)	100 µL × 1 vial (0.5 µg/mL)	Operate as per the recommended dilution procedure
Anti-Benzonase (enzyme-labeled antibody)	15 mL× 1 bottle	Ready-to-use
Sample Diluent Buffer	30 mL× 1 bottle	Ready-to-use
20× Wash Buffer	30 mL× 1 bottle	Operate as per the recommended dilution procedure
Color Reagent A	8 mL× 1 vial	Ready-to-use
Color Reagent B	8 mL× 1 vial	Ready-to-use
Stop Solution	15 mL× 1 bottle	Ready-to-use
Plate Sealer	3 pieces	Ready-to-use
Instructions for Use	1 copy	/

Notes: Reagents should be stored at 2 ~ 8℃; Color Reagents A and B should be protected from light during storage.

Storage conditions and shelf life

The shelf life of unopened kits is 12 months when stored at 2 ~ 8℃.

Apparatus to be prepared by the user:

1. Plate reader, thermostate plate shaker or thermostate incubator, plate washer
2. High-precision pipette and disposable tips (0.5 ~ 10 µL, 10 ~ 100 µL, 30 ~ 300 µL, and 100 ~ 1000 µL).
3. Deionized water, blotting paper, EP tubes

Reagent preparation

1. 1× Wash Buffer: Take 1 portion of Wash Buffer (20×), and add 19 times the volume of deionized water to prepare the Wash Buffer at working concentration (1×). If there are crystals in the Wash Buffer (20×), shake gently at room temperature or in a 37℃ water bath, and dilute after the crystals are completely dissolved. Unused Wash Buffer (20×) should be stored at 2 ~ 8℃.

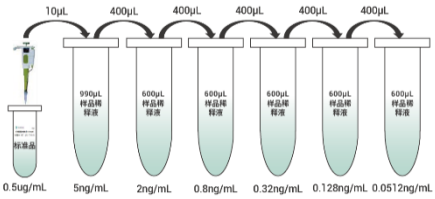
2. Preparation of standard: Dilute the standard to 5 ng/mL with the Sample Diluent Buffer, and prepare the standard by 2.5fold dilution.

3. Preparation of substrate solution: Mix Color Reagents A and B at equal volume at 10 minutes before use, and the operation should be performed at dark environment. Make sure that the substrate solution is not contaminated. Do not use if the substrate solution turns blue after mixing.

Test procedures

1. Equilibrate the temperature of each component in the kit to room temperature for 30 minutes. Take out required strip plates from aluminum foil bags already equilibrated to room temperature, and label the strip plate sequence with a marker. Seal remaining strip plates with a plate sealer, put them back to the aluminum foil bag, then seal the bag, and store at 2 ~ 8℃.

2. Preparation of standard: Dilute the standard to 5 ng/mL with the Sample Diluent Buffer, and prepare the standard by 2.5-fold dilution, as shown in the figure on the right.



3. Sample incubation: Set the standard wells, blank wells, and test sample wells, respectively. Add standards at different concentrations (in sequence), Sample Diluent Buffer, and test sample to standard wells, blank wells, and test sample wells, respectively (100 μL/well), seal the plate with a plate sealer, and incubate at 37℃ for 1 hour.

4. Plate washing: Discard liquid in the wells. Wash the plate for 3 times with 1× Wash Buffer (250 μL/well), and pat dry the residual liquid in test sample wells. (After adding the Wash Buffer each time, if the plate is to be washed manually, allow the plate to stand for 1 minute after adding the Wash Buffer and shake gently; if the plate is to be washed with a plate washer, shake the plate gently for 5 seconds after adding the Wash Buffer.)

5. Incubation of enzyme-labeled antibody: Add 100 μL of enzyme-labeled antibody into each well, seal the plate with a plate sealer, and incubate at 37℃ for 1 hour.

6. Plate washing: Same as Step 4.

7. Color development: Add the pre-prepared substrate solution into the plate (100 μL/well) and mix well, seal the plate with a plate sealer, and incubate at 37℃ for 15 minutes while being protected from light.

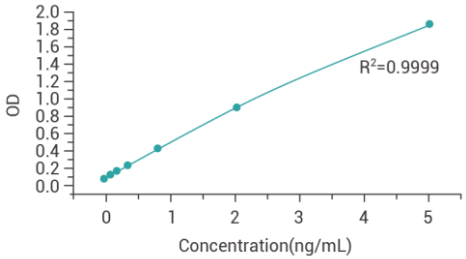
8. Reaction termination: Add stop solution at 100 μL/well.

9. Reading: Measure the OD values at 450 nm and 630 nm with a plate reader. The measurement should be completed within 20 minutes after reaction termination.

Results process

Plot the curve with the OD value ($OD_{450\,nm}$ - $OD_{630\,nm}$) of the nuclease standard as the dependent variable Y and the standard concentration as the independent variable X. The 4-parameter logistic fitting equation is recommended: Y = ((A - D)/(1 + (x/C)^B) + D. Substitute the OD value of test sample ($OD_{450\,nm}$ - $OD_{630\,nm}$) to the equation to calculate the nuclease content in the test sample).

Standard curve



Assay result

Standard concentration (ng/mL)	OD value (1)	OD value (2)	Mean value
5	1.925	1.774	1.849
2	0.917	0.865	0.891
0.8	0.447	0.441	0.444
0.32	0.228	0.209	0.218
0.128	0.146	0.137	0.141
0.0512	0.114	0.107	0.110

Precautions

1. This kit is for *in vitro* detection only, and may not be used for clinical diagnosis.
2. The kit must be used within the shelf life.
3. All components in the kit must be equilibrated to room temperature (20 ~ 25℃) before use.
4. Fully mix each components of the kit before use. When patting to dry the plates after washing, protect the strip plates from falling.
5. The optimal assay results may only be achieved by strictly following the instructions and using only the reagents provided in the kit.
6. Please timely replace reagent troughs and pipette tips when loading different samples and performing different steps, so as to avoid cross contamination.
7. The final assay results are closely related to reagent effectiveness, the operations of analysts, and the test environment.
8. Our company is only responsible for the kits themselves, and will not be responsible for the sample consumption caused by kits during use. Users should fully consider the possible sample consumption before operation, and should reserve sufficient sample size.

Safety reminder

1. The stop solution in this kit is acidic, thus extra attention should be paid during operation.
2. All biological samples may cause potential biosafety risks, therefore, users must strictly follow local laws and regulations when handling and disposing of the samples.
3. For safety concern, the operators should wear personal protective equipment such as lab coat, gloves, mask, and safety glasses.

Problem analysis

In case of any problems with the assay results, please take photos of the color development results in a timely manner, properly store the unused strip plates and reagents, and contact us for technical support. Alternatively, the user may refer to the following information to identify the cause.

Problem description	Possible reason	Corresponding measure
Poor gradient of standard curve	Inaccurate liquid pipetting or addition	Check the pipette and tips
	Insufficient plate washing	Ensure the number of plate washing times and the volume of Wash Buffers for each well
Very weak or no color	Excessively short incubation duration	Ensure adequate incubation duration
	Incorrect experimental temperature	Adopt the recommended incubation temperature
	Inadequate reagent volume or missed addition of reagent	Check procedures of liquid pipetting and addition, so as to ensure that all reagents are added in specified order and at adequate volume.
Low OD value	Incorrect setting of plate reader	Check the wavelength and filter device on the plate reader
		Preheat the plate reader in advance before reading
Large CV	Improper liquid addition	Check procedure of liquid addition
	Contaminations at plate bottom	Check the plate bottom for any remaining liquid or fingerprints
	Foreign matters or air bubbles in the wells	Make sure there are no foreign matters in the wells before loading, and make sure there are no air bubbles in the wells after loading
High background value	Insufficient plate washing	Wash the plate as per the method recommended in the instructions for use
		If using an automatic plate washer, please check all the liquid addition ports and discharging outlets for blockages
		If washing the plate manually, increase the number of plate washing times as appropriate.
	Contaminated Wash Buffer	Prepare fresh Wash Buffer
Low sensitivity	Improper storage of the kit	Store relevant reagents as per the instructions for use.

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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