



TECHNICAL MANUAL

Benzonase Nuclease PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00004
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Benzonase Nuclease PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Assay Range:

0.512 ng/mL – 5 ng/mL

Limit of quantification:

-

Limit of detection:

-

Detection Method:

Sandwich

Sample Type:

Biological samples

Precision:

-

2. Storage & Expiry

Assay Genie ELISA Kits are shipped on ice packs. Please store this ELISA Kit as indicated in section 4. Validity for 12 months . Date of expiration is on the ELISA Box label.

3. Product Description

The Benzonase Nuclease PharmaGenie ELISA Kit is designed for the quantitative detection of residual nuclease in intermediates, semi-finished, and finished biological products. This kit is particularly valuable in biopharmaceutical manufacturing and quality control, where it is essential to confirm the effective removal of Benzonase nuclease used during cell lysis and nucleic acid degradation steps. Typical applications include testing in-process samples, purification intermediates, and final drug substance to ensure product safety, purity, and compliance with regulatory requirements.

This highly sensitive assay utilizes a double-antibody sandwich ELISA to detect trace amounts of Benzonase nuclease with accuracy and reliability. The 96-well microplate is pre-coated with a nuclease-specific capture antibody, enabling immobilization of the target enzyme. Standards and test samples are then added, followed by an HRP-conjugated detection antibody to form an antibody–nuclease–antibody sandwich complex. After washing to remove unbound components, a chromogenic substrate is added. Under HRP catalysis, the substrate initially develops a blue colour, which transitions to yellow after the stop solution is applied. The optical density (OD) is measured at 450 nm, and the nuclease concentration is calculated from the standard curve.

4. Kit Contents

No	Component Name	Size	Preparation	Storage
1	Benzonase Coated Plate	8 wells x 12 strips	Ready-to-use	Store 2 ~ 8°C
2	Benzonase Standard (standard)	100 µL x 1 vial (0.5 µg/mL)	Operate as per the recommended dilution procedure	
3	Anti-Benzonase (enzyme-labelled antibody)	15 mL x 1 bottle	Ready-to-use	
4	Sample Diluent Buffer	30 mL x 1 bottle	Ready-to-use	
5	20x Wash Buffer	30 mL x 1 bottle	Operate as per the recommended dilution procedure	
6	Colour Reagent A	8 mL x 1 vial	Ready-to-use	Store 2 ~ 8°C (Protect from light)
7	Colour Reagent B	8 mL x 1 vial	Ready-to-use	
8	Stop Solution	15 mL x 1 bottle	Ready-to-use	Store 2 ~ 8°C
9	Plate Sealer	3 pieces	Ready-to-use	-
10	Technical Manual	1 copy		-

Additional materials required:

1. 37°C incubator.
2. Plate Reader with 450nm filter.
3. Precision pipettes and disposable pipette tips.
4. Distilled water.
5. Disposable tubes for sample dilution.
6. Absorbent paper.

5. Precautions

1. Store all reagents according to the instructions on the product label. Before use, allow all reagents to equilibrate to room temperature.
2. Before opening the secondary packaging, bring the pre-coated strip plates to room temperature. Return any unused strips immediately to the original packaging and reseal tightly. Store unused plates at 4°C for up to one month. All other unused reagents should be properly sealed or covered.
3. The volumes of the standard, biotinylated antibody, and enzyme conjugate are small. Perform a quick centrifugation prior to use to ensure that any liquid adhering to the tube walls or caps collects at the bottom.
4. Always use disposable pipette tips during the assay to prevent cross-contamination.
5. Inspect all kit components before use. To ensure accurate results, mix thoroughly when preparing dilutions, loading samples, or adding stop solution.
6. During the washing steps, after removing Wash Buffer, tap the plate dry on clean absorbent paper until no residual droplets or watermarks are visible. Do not insert tissue directly into the wells.
7. The TMB substrate is photosensitive, protect it from prolonged light exposure. Avoid contact with metal surfaces, as this may interfere with the reaction.
8. This kit is intended for single use and should be used within its stated shelf life.

6. Sample Preparation

Due to the inherent variability of biological samples and the specific requirements of individual assays, users are advised to optimize protocols in accordance with their own experimental conditions. Samples may be tested directly with this ELISA or diluted as necessary, based on experimental objectives and the physicochemical characteristics of the sample matrix.

Note: For information regarding validation data in specific samples, please contact our Technical Support Team at techsupport@assaygenie.com.

7. Reagent Preparation

1. **1X Wash Buffer:** Take 1 portion of Wash Buffer (20x), and add 19 times the volume of deionized water to prepare the Wash Buffer at working concentration (1x). If there are crystals in the Wash Buffer (20x), shake gently at room temperature or in a 37°C water bath, and dilute after the crystals are completely dissolved. Unused Wash Buffer (20x) should be stored at 2 ~ 8°C.
2. **Preparation of standard:** Prepare the standard curve using a series of seven sequential dilutions starting from a 0.5 µg/mL stock standard solution. Pipette 10 µL of the 0.5 µg/mL standard into 990 µL of sample dilution buffer to obtain the first standard at 5 ng/mL. Transfer 400 µL of the 5 ng/mL solution into 600 µL of sample dilution buffer to prepare the second standard at 2 ng/mL. Continue performing 1:2.5 serial dilutions by transferring 400 µL from the previous tube into 600 µL of fresh sample dilution buffer for each step. Prepare the remaining standards sequentially to achieve the following concentrations: 5 ng/mL, 2 ng/mL, 0.8 ng/mL, 0.32 ng/mL, 0.128 ng/mL and 0.0512 ng/mL. Mix each dilution thoroughly before proceeding to the next step. The prepared standards are used to generate the ELISA standard curve for quantitative analysis of test samples.
3. **Preparation of substrate solution:** Mix Colour 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.

8. Assay Procedure

1. **Equilibrate Reagents:** Bring 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°C.
2. **Setup Plate:** Set the standard wells, blank wells and test sample wells, respectively. Recommended to assay samples in duplicate.
3. **Add Standard, Sample and Blanks:** 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).
4. **First Incubation:** Seal the plate with a plate sealer, and incubate at 37°C for 1 hour.
5. **Washing:** Discard liquid in the wells. Wash the plate for 3 times with 1x 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.)
6. **Incubation of enzyme-labelled antibody:** Add 100 µL of enzyme-labelled antibody into each well, seal the plate with a plate sealer, and incubate at 37°C for 1 hour.
7. **Washing:** Same as Step 5.
8. **Colour 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°C for 15 minutes while being protected from light.
9. **Reaction termination:** Add stop solution at 100 µL/well.
10. **Signal 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.

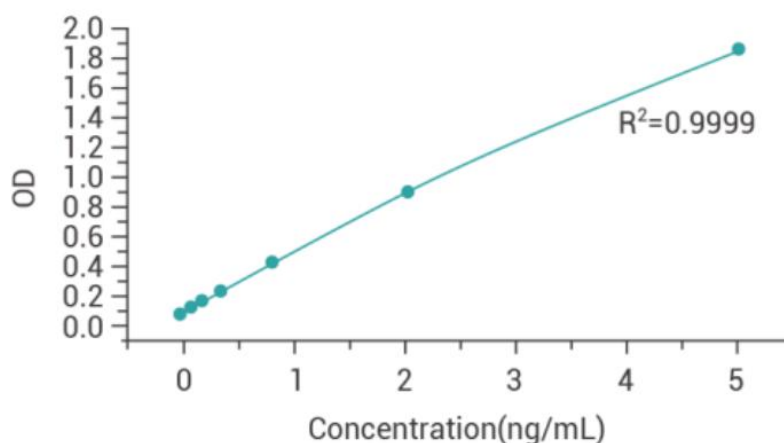
9. Data Analysis

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

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

Std 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

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).



10. ELISA Troubleshooting

Problem	Possible Causes	Solutions
Standard curve without signal	Incorrect reagent order; Mixed components from different kits; Missing reagents.	Ensure correct reagent order and use components from the same kit. Verify all reagents are added.
Overflow OD	Mixed components from different kits; Over-concentrated working solution	Use correct components and prepare solutions at recommended concentrations.
Poor standard curve	Incorrect curve fitting model.	Try alternative curve fitting models.
Samples without signal	Sample concentration too low; Incompatible buffer; Incorrect preparation; Sample degradation or excessive freeze-thaw.	Reduce dilution or concentrate sample. Check buffer compatibility and follow proper preparation and storage.
High CV%	Precipitate formation; Unclean plate; Foaming; Uneven washing; Incomplete reagent mixing; Pipetting inconsistency.	Dilute samples if needed, avoid foaming, ensure uniform washing, mix reagents thoroughly, and use calibrated pipettes.
Low standard signal	Improperly reconstituted standards; Degraded standards; Incorrect pipetting; Expired kit; Improper storage; Over-dried wells.	Reconstitute standards properly, use fresh kits, follow storage recommendations, and prevent wells from drying.
Slow colour development	TMB not equilibrated; Incorrect microplate reader wavelength; Over-washing.	Pre-warm TMB (30 min at 37°C), confirm correct wavelength (450 nm), and follow recommended washing times.
High background	Insufficient washing; Contaminated wash buffer; Excess detection reagents; Delayed reading; TMB exposed to light.	Wash adequately, prepare fresh wash buffer, use correct reagent amounts, read results promptly, and incubate TMB in the dark.

Notes:

Assay Genie 100% money-back guarantee!

If you are not satisfied with the quality of our products and our technical team cannot resolve your problem, we will give you 100% of your money back.

