Benzonase Nuclease ELISA Kit

Benzonase Nuclease ELISA Kit is an Enzyme Immunoassay kit for the quantification of Benzonase Nuclease in biopharmaceuticals.

Catalog number: ARG83099

Package: 96 wells

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INTRODUCTION

Benzonase Nuclease ELISA Kit is designed for the quantitative detection of residual nuclease content in intermediates, semifinished products and finished products of various biological products by using a double-antibody sandwich method..

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for Benzonase Nuclease has been pre-coated onto a 96 well microplate. Benzonase Nuclease antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti- Benzonase Nuclease antibody is added and binds to Benzonase Nuclease antigen captured by the first antibody. After washing away any unbound substances, a HRP Conjugate mouse anti-FITC antibody is added and incubation. After washing away any unbound substances, the TMB substrate is added to the wells and color develops in proportion to the amount of Benzonase Nuclease bound in the initial step. The color development is stopped by the addition of stop solution and the intensity of the color is measured at a wavelength of 450 nm. The concentration of Benzonase Nuclease in the samples is then determined by comparing the O.D of samples to the standard curve.

MATERIALS PROVIDED & STORAGE INFORMATION

Use the kit before expiration date.

Component	Quantity	Storage information
Antibody Coated Microplate	8 X 12 strips	4°C
Standard	300 µL (0.5 µg/mL)	4°C
Antibody Conjugate	15 mL (ready to use)	4°C
Diluent Buffer	30 mL (ready to use)	4°C
20X Wash Buffer	30 mL	4°C
TMB Substrate A	8 mL	4°C (protect from light)
TMB Substrate B	8 mL	4°C (protect from light)
STOP Solution	15 mL (ready to use)	4°C
Plate sealer	3 pieces	4°C

MATERIALS REQUIRED BUT NOT PROVIDED

- Microplate reader capable of reading at 450 nm
- Deionized or distilled water
- Pipettes and pipette tips
- Multichannel micropipette reservoir
- Microtiter plate washer

TECHNICAL NOTES AND PRECAUTIONS

- Wear protective gloves, clothing, eye, and face protection especially while handling blood or body fluid samples.
- Upon received, store at 2-8°C at all times.
- Prior to beginning the assay procedure, bring all reagents and required number of strips to room temperature (20-25°C).
- Unused wells must be stored at 2-8 °C in the sealed foil pouch and used in the frame provided.
- All reagents must be mixed without foaming and briefly spin down the all vials before use.
- If crystals are observed in the 20X Wash Buffer or Diluent Buffer warm to 37°C until the crystals are completely dissolved.
- Minimize lag time between wash steps to ensure the plate does not become completely dry during the assay.
- Ensure complete reconstitution and dilution of reagents prior to use.
- Take care not to contaminate the mixed TMB Substrate. Do not expose the mixed TMB solution to glass, foil or metal. If the solution is blue before use, DO NOT USE IT.
- All reagents must be mixed without foaming before use.
- Change pipette tips between the addition of different reagent or samples.
- Taping the well strips together with lab tape can be done as an extra precaution to avoid plate strips from coming loose during the procedure.

REAGENT PREPARATION

- 1X Wash Buffer: Dilute 20X Wash Buffer into distilled water to yield 1X Wash Buffer. The 1X Wash Buffer is stable for up to 4 weeks at 2-8°C. Mix well before use.
- TMB Substrate Mixture: It is recommended to prepare this reagent immediately prior to use and use it within 10 min after preparation. Mix TMB Substrate A with equal volume TMB Substrate B, protect from light.
- Standards: The Diluent Buffer serves as zero standard (0 ng/ml), and the rest of the standard serial dilution can be diluted as according to the suggested concentration below: 5 ng/ml, 2 ng/ml, 0.8 ng/ml, 0.32ng/ml, 0.128 ng/ml, 0.0512 ng/ml. DO NOT reuse the reconstituted standard.

  Dilute Benzonase Nuclease standard as according to the table below:

Standard	Benzonase Nuclease Conc.	µl of Diluent Buffer	µl of standard
S6	5 ng/ml	990 µl	10 µl (stock)
S5	2 ng/ml	600 µl	400 µl(S7)
S4	0.8 ng/ml	600 µl	400 µl(S6)
S3	0.32 ng/ml	600 µl	400 µl(S5)
S2	0.128 ng/ml	600 µl	400 µl(S4)
S1	0.0512 ng/ml	600 µl	400 µl(S3)
S0	0 ng/ml	1000 µl	0

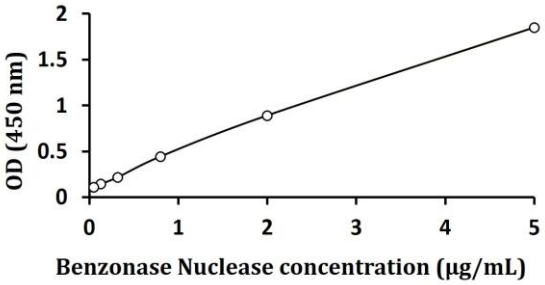
ASSAY PROCEDURE

All materials should be equilibrated to room temperature (RT, 20-25°C) before use.

1. Add **100 µL** of the **Standard** or **Samples** to the Antibody Coated microplate.
2. Cover the plate and incubate for **1 hours** at **37°C**.
3. Aspirate each well and wash, repeating the process 2 times for a **total 3 washes.** Wash by filling each well with **1X Wash Buffer (250 µL)** using a squirt bottle, manifold dispenser, or autowasher. Keep the wash buffer in the wells for 30-60 sec before remove. Complete removal of liquid at each is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating, decanting or blotting against clean paper towels.
4. Add **100 µL** of **1X Antibody Conjugate** to each wells.
5. Cover the plate and Incubate for **1 hour** at **37°C**.
6. Aspirate each well and **wash as step 3**.
7. Add **100 µL** of **TMB Substrate Mixture** to each well. Cover the plate and incubate for **10-20 minutes** at **37°C**.
8. Add **100 µL** of **Stop Solution** to each well, gently tap the plate to mix well.
9. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 20 minutes** after adding the stop solution.

EXAMPLE OF TYPICAL STANDARD VALUES

The following data is for demonstration only and cannot be used in place of data generations at the time of assay.



CALCULATION OF RESULTS

1. Calculate the average absorbance values for each set of standards and samples.
2. To obtain more accurate results, more dilution points can be used when generating standard curves. 4 Parameter Logistics is the preferred method for the result calculation. Other data reduction functions may give slightly different results.
3. arigo provides GainData®, an in-house development ELISA data calculator, for ELISA data result analysis. Please refer our GainData® website for details. (https://www.arigobio.com/elisa-analysis)
4. If the samples have been diluted, the concentration read from the standard curve must be further converted by the appropriate dilution factor according to the sample preparation procedure.