DNase I ELISA Kit

DNase I ELISA Kit is an Enzyme Immunoassay kit for the quantification of DNase I in biopharmaceuticals.

Catalog number: ARG83105

Package: 96 wells

TABLE OF CONTENTS

MANUFACTURED BY:

Arigo Biolaboratories Corporation

Address: No. 22, Ln. 227, Gongyuan Rd., Hsinchu City 300, Taiwan

Phone: +886 (3) 562 1738

Fax: +886 (3) 561 3008

Email: info@arigobio.com

INTRODUCTION

DNase I ELISA kit is designed for the quantitative detection of residual Dnase I content added in RNApharmaceuticals processes by using a double-antibody sandwich method.

PRINCIPLE OF THE ASSAY

DNase I ELISA kit employs the quantitative sandwich enzyme immunoassay technique. A capture antibody specific for DNase I has been pre-coated onto a 96 well microplate. DNase I antigen present in the sample or standard binds to the antibodies adsorbed on the plate; a FITC-conjugated mouse anti- DNase I antibody is added and binds to DNase I 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 DNase I 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 DNase I 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	30 µL (100 µg/mL)	-20°C
Sample Diluent Buffer	60 mL (ready to use)	4°C
100X Antibody Conjugate	150 µL	-20°C
Antibody Conjugate Diluent Buffer	12 mL (ready to use)	4°C
100X HRP- Streptavidin Solution	150 µL	-20°C
HRP- Streptavidin Diluent Buffer	12 mL (ready to use)	4°C
20X PBST Wash Buffer	50 mL	4°C
TMB Substrate	11 mL (ready to use)	4°C (protect from light)
STOP Solution	7 mL (ready to use)	4°C
Plate sealer	5 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

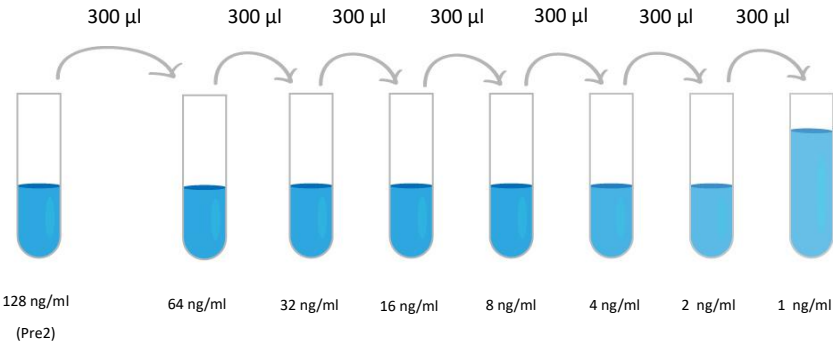
TECHNICAL NOTES AND PRECAUTIONS

- 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 PBST 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.
- 1X Antibody Conjugate: It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute 100X Antibody Conjugate Mixture concentrate into Antibody Conjugate Diluent Buffer to yield 1X Antibody Conjugate.
- 1X HRP-Streptavidin Solution: It is recommended to prepare this reagent immediately prior to use and use it within 20 min after preparation. Dilute 10X HRP-Streptavidin Solution into HRP- Streptavidin Diluent Buffer to yield 1X HRP-Streptavidin Solution.
- 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: 64 µg/ml, 32 µg/ml, 16 µg/ml, 8 µg/ml, 4 µg/ml, 2 µg/ml, 1 µg/ml. DO NOT reuse the reconstituted standard.



Dilute DNase I standard as according to the table below:

Standard	DNase I Conc.	µl of Diluent Buffer	µl of standard
Pre 1	10,000 ng/ml	5 µl	45 µl (Stock)
Pre 2	128 ng/ml	8 µl	617 µl (Pre1)
S7	64 ng/ml	300 µl	300 µl (Pre2)
S6	32 ng/m	300 µl	100 µl(S7)
S5	16 ng/m	300 µl	100 µl(S6)
S4	8 ng/m	300 µl	100 µl(S5)
S3	4 ng/m	300 µl	100 µl(S4)
S2	2 ng/m	300 µl	100 µl(S3)
S1	1 ng/m	300 µl	100 µl(S2)
S0	0 ng/ml	300 µl	0

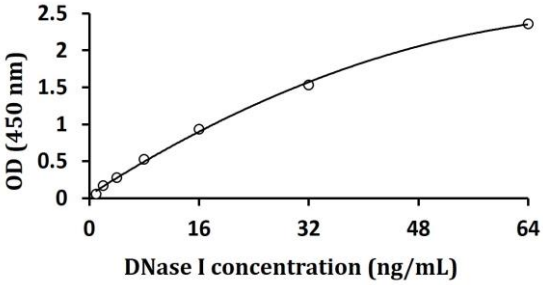
ASSAY PROCEDURE

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 **1X HRP-Streptavidin Solution** to each well.
8. Cover the plate and incubate for **1 hour** at **room temperature**.
9. Aspirate each well and **wash as step 3**.
10. Add **100 µL** of **TMB Substrate** to each well. Cover and incubate for **10 minutes** at **room temperature** in the dark.
11. Immediately Add **50 µL** of **Stop Solution** to each well, gently tap the plate to mix well. The color of the solution should change from blue to yellow.
12. Read the OD with a microplate reader at **450 nm** immediately. It is recommended reading the absorbance **within 10 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.