TECHNICAL MANUAL

DNase I Residual Contamination PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00009
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 08/2025

DNase I Residual Contamination PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

Table of Contents

1. Key Features

Assay Range:

1-64 ng/mL

Limit of quantification:

1 ng/mL

Limit of quantification:

0.5 pg/mL

Detection Method:

Sandwich

Sample Type:

Biological samples

Precision:

CV%≤10%, RE%≤±15%

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

he DNase I Residual Contamination PharmaGenie ELISA Kit enables the quantitative detection of residual DNase I in biological products. DNase I is frequently used in biopharmaceutical manufacturing to degrade unwanted DNA during cell lysis, protein purification, and viral vector production. However, incomplete removal of DNase I can compromise product safety by degrading therapeutic nucleic acids or interfering with downstream molecular assays. Regulatory authorities require strict monitoring of DNase I clearance to ensure product integrity, efficacy, and patient safety.

This high-sensitivity assay employs a double-antibody sandwich enzyme immunoassay for precise and reproducible detection. Microplate wells are pre-coated with DNase I-specific monoclonal capture antibodies. Standards and test samples are added and incubated, allowing any DNase I present to bind to the immobilized antibodies. After washing to remove unbound material, an anti-DNase I monoclonal detection antibody is added, followed by an H+L secondary antibody conjugated to an enzyme, forming an antibody–antigen–antibody–enzyme complex.

The TMB substrate is then added, producing a color change proportional to the DNase I concentration. Absorbance is measured at 450 nm, and concentrations are calculated from the standard curve, providing a sensitive, quantitative method for verifying DNase I removal in compliance with regulatory guidelines.

4. Kit Contents

No	Component Name	Size	Preparation	Storage
1	DNase I Coated Plate	8 wells x 12 strips	Ready-to-use	Store 2 ~ 8°C
2	Anti-DNase I (detection antibody)	150 µL x 1 vial	1:100, dilute with Antibody Diluent Buffer	Store -18°C
3	Streptavidin HRP (enzyme conjugate)	150 µL x 1 vial	1:100, dilute with Enzyme Conjugate Diluent Buffer	
4	DNase I Standard (standard)	30 µL x 1 vial (100 µg/mL)	Operate as per the recommended dilution procedure	
5	Sample Diluent Buffer	60 mL x 1 bottle	Ready-to-use	Store 2 ~ 8°C (Protect from light)
6	Antibody Diluent Buffer	12 mL x 1 bottle	Ready-to-use	
7	Enzyme Conjugate Diluent Buffer	12 mL× 1 bottle	Ready-to-use	
8	20x PBST Wash Buffer	50 mL x 1 bottle	1: 20, dilute with deionized water	
9	TMB Substrate	11 mL x 1 bottle	Ready-to-use	
10	Stop Solution	7 mL x 1 bottle	Ready-to-use	
13	Plate Sealer	5 piece	-	-
14	Technical Manual	1 copies	-	-

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 PBST Wash Buffer:** Calculate the volume of working solution required for the test, transfer an appropriate amount of 20 x PBST Wash Buffer, dilute with deionized water in a ratio of 1:20, and mix well for later use.
2. **Detection antibody and enzyme conjugate working buffer:** Calculate the volume of working solution required for the test, transfer an appropriate amount of biotin antibody or enzyme conjugate, dilute with corresponding diluent buffer in a ratio of 1:100, and mix well for later use.
3. **Preparation of standard:** The standard and test samples should be diluted with the Diluent Buffer. Follow dilutions as below. Note: Store the first intermediate gradient (Pre-1) of the standard at -20°C for 1-7 days.

Vial	Std [pg/mL]	Std vol (µL)	Diluent Buffer (µL)	Total Vol (µL)	Final conc [pg/mL]	Remaining volume (µL)
Pre - 1	100000	5	45	50	10000	490
Pre - 2	10000	8	617	625	128	475
8	128	300	300	600	64	80
7	64	300	300	600	32	300
6	32	300	300	600	16	300
5	16	300	300	600	8	300
4	8	300	300	600	4	300
3	4	300	300	600	2	300
2	2	300	300	600	1	600
1	-	-	300	300	0	300

8. Assay Procedure

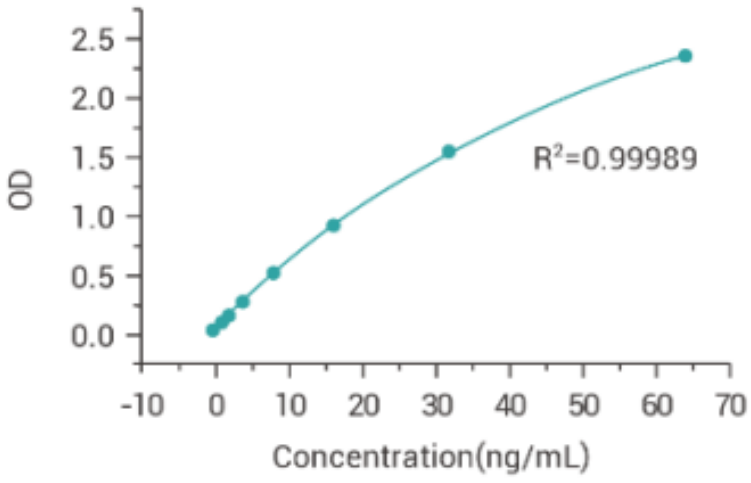
1. **Equilibrate Reagents:** Bring each component in the kit to room temperature for 30 minutes.
2. **Setup Plate:** Set the standard wells, blank wells and test sample wells, respectively. Recommended to assay samples in duplicate.
3. **Add Standard, Sample and Blanks:** Add standard, sample dilution working buffer, and negative control into respective wells at 100 µL/well.
4. **First Incubation:** Seal the microplate with microplate sealer and incubate in a 37°C constant temperature shaking incubator at 200-300 rpm for 60 minutes.
5. **Washing:** Discard the liquid in each well, and fill the wells with 1× PBST Wash Buffer (300 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 3 times, and pat the plate dry on tissue after each washing.
6. **Addition of detection antibody working buffer:** Add 100 µL of detection antibody working buffer to each well.
7. **Second Incubation:** Seal the microplate with microplate sealer and incubate in a 37°C constant temperature shaking incubator at 200-300 rpm for 60 minutes.
8. **Addition of enzyme conjugate working buffer:** Add 100 µL of enzyme conjugate working buffer to each well.
9. **Washing:** Repeat step 5.
10. **Colour development:** Add 100 µL of TMB Substrate to each well, gently shake to mix well, seal the plate with a plate sealer, and place the plate at 25℃ for 10 minutes for color development reaction.
11. **Reaction termination**: Add 100 µL of stop solution to each well and immediately perform the detection.
12. **Signal Reading**: Measure the OD values at 450 nm and 630 nm with a plate reader. The measurement should be completed within 30 minutes after reaction termination.

9. Data Analysis

The 4-parameter fitting method is recommended for the linear fitting and calculation of the product. (1) OD processing of the standard curve (the following example is provided as reference only, and the results from actual detection shall prevail).

Standard concentration (pg/mL)	OD value (1)	OD value (2)	Mean value
64	2.373	2.347	2.360
32	1.520	1.546	1.533
16	0.933	0.934	0.9335
8	0.518	0.536	0.527
4	0.259	0.291	0.275
2	0.166	0.168	0.167
1	0.115	0.109	0.112
0	0.057	0.049	0.053

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.