TECHNICAL MANUAL

dsRNA Residual Contamination PharmaGenie ELISA Kit

- **SKU CODE:** AEGE00012
- **SIZE:** 96T
- **DETECTION PRINCIPLE:** Sandwich
- **RUO:** Research-Use-Only

Revised: 08/2025

dsRNA Residual Contamination PharmaGenie ELISA Kit

Please read entire manual carefully before starting experiment.

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1. Key Features

Assay Range:

The detection linear range of unmodified, pUTP-modified dsRNA is 0.0156-0.5 pg/μL

The detection linear range of N1-Me-pUTP-modified dsRNA is 0.0312-1 pg/μL

The detection linear range of 5-OMe-UTP-modified dsRNA is 0.0625-1 pg/μL

Limit of quantification:

The LoD of unmodified, pUTP-modified and N1-Me-pUT

The LoD of 5-OMe-UTP-modified dsRNA is 0.01 pg/μ

Limit of quantification:

The LoQ of unmodified, pUTP-modified dsRNA is 0.0156 pg/μL

The LoQ of N1-Me-pUTP-modified dsRNA is 0.0312 pg/μL The LoQ of 5-OMe-UTP-modified dsRNA is 0.0625 pg/μL

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

The Double-Stranded RNA (dsRNA) Residual Contamination PharmaGenie ELISA Kit enables the quantitative detection of dsRNA ≥60 base pairs in biological products, regardless of nucleotide sequence. dsRNA can arise during viral replication, in vitro transcription, or certain recombinant expression processes, and is a known immunostimulatory molecule that can activate innate immune responses via pattern recognition receptors. If not effectively removed, residual dsRNA in therapeutic products—such as mRNA vaccines, viral vectors, or recombinant proteins—can trigger inflammation, reduce efficacy, or cause adverse immune reactions. Regulatory authorities require sensitive detection of dsRNA to ensure product safety and quality.

This high-sensitivity assay uses a double-antibody sandwich method coupled with a streptavidin–biotin system for accurate and reproducible detection. Microplate wells are coated with anti-dsRNA capture antibodies. Samples are added, incubated, and washed, followed by a biotinylated detection antibody to form an antibody–antigen–antibody complex. After an additional wash step, a streptavidin–HRP conjugate is added to complete the detection system.

Following thorough washing, TMB substrate is introduced, producing a blue colour under HRP catalysis that turns yellow upon addition of the stop solution. The optical density (OD) is measured at 450 nm, and concentrations are calculated from a standard curve, providing a robust method for confirming dsRNA clearance in compliance with stringent quality control requirements.

4. Kit Contents

No	Component Name	Size	Preparation	Storage
1	Coated microtiter plate	8 × 12 plate strips	Ready-to-use	Store the kit at 2°C-8 °C
2	Biotinylated detection antibody (100×)	120 µL × 1 tube	Dilute 100-fold with diluent	
3	Streptavidin–HRP (100×)	120 µL × 1 tube	Dilute 100-fold with diluent	
4	Diluent	30 mL × 1 bottle	Ready-to-use	
5	Chromogenic solution	12 mL × 1 bottle	Ready-to-use	
6	Stop solution	6 mL × 1 bottle	Ready-to-use	
7	20× wash solution	40 mL × 1 bottle	Dilute with purified water at 1:19 (v/v) to prepare wash working solution	
8	dsRNA Standard (unmodified, 5 ng/µL)	15 µL × 1 tube	Dilute with STE buffer to required concentration	
9	dsRNA Standard (pUTP-modified, 5 ng/µL)	15 µL × 1 tube	Dilute with STE buffer to required concentration	
10	dsRNA Standard (N1-Me-pUTP-modified, 5 ng/µL)	15 µL × 1 tube	Dilute with STE buffer to required concentration	
11	dsRNA Standard (5-OMe-UTP-modified, 5 ng/µL)	15 µL × 1 tube	Dilute with STE buffer to required concentration	

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. **20X Wash Solution:** Calculate the volume of working solution required for the test, transfer an appropriate amount of 20X Wash Solution, dilute with deionized water in a ratio of 1:19, and mix well for later use.
2. **100× HRP-SA and 100× biotinylated detection antibody adetection antibody:** Centrifuge the antibody tube, HRP-SA tube and standard tube at 1000 rpm for 30 s before use to avoid residual reagents on the tube wall and cap. 100× biotinylated detection antibody and 100× streptavidin-HRP are diluted 100-fold with diluent before use.
3. **Preparation of standard:**
   - Dilute unmodified, pUTP-modified dsRNA Standards to 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0.0156, and 0 pg/μL with STE buffer.
   - Dilute N1-Me-pUTP-modified dsRNA Standards to 2, 1, 0.5, 0.25, 0.125, 0.0625, 0.0312, 0 pg/μL with STE buffer.
   - Dilute 5-OMe-UTP-modified dsRNA Standards to 4, 2, 1, 0.5, 0.25, 0.125, 0.0625, 0 pg/μL with STE buffer.

a. Unmodified, pUTP-modified

	Final Concentration (pg/µL)	STE Buffer	Working Standard
	100	49 µL	1 µL of 5 ng/µL standard
A	1	495 µL	5 µL of 100 pg/µL solution
B	0.5	250 µL	250 µL of Solution A
C	0.25	250 µL	250 µL of Solution B
D	0.125	250 µL	250 µL of Solution C
E	0.0625	250 µL	250 µL of Solution D
F	0.0312	250 µL	250 µL of Solution E
G	0.0156	250 µL	250 µL of Solution F
H	0	250 µL	/

b. N1-Me-pUTP-modified

	Final Concentration (pg/µL)	STE Buffer	Working Standard
	100	49 µL	1 µL of 5 ng/µL standard
A	2	490 µL	10 µL of 100 pg/µL solution
B	1	250 µL	250 µL of Solution A
C	0.5	250 µL	250 µL of Solution B
D	0.25	250 µL	250 µL of Solution C
E	0.125	250 µL	250 µL of Solution D
F	0.0625	250 µL	250 µL of Solution E
G	0.0312	250 µL	250 µL of Solution F
H	0	250 µL	/

c. 5-OMe-UTP-modified

	Final Concentration (pg/µL)	STE Buffer	Working Standard
	100	49 µL	1 µL of 5 ng/µL standard
A	4	480 µL	20 µL of 100 pg/µL solution
B	2	250 µL	250 µL of Solution A
C	1	250 µL	250 µL of Solution B
D	0.5	250 µL	250 µL of Solution C
E	0.25	250 µL	250 µL of Solution D
F	0.125	250 µL	250 µL of Solution E
G	0.0625	250 µL	250 µL of Solution F
H	0	250 µL	/

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. ***Note:*** *When it is not possible to determine the dsRNA content in the sample to be tested, dilutions shall be made with the STE buffer for the detection to avoid excessive content and inability to read valid values.*
4. **First Incubation:** Seal the microplate with microplate sealer and incubate at room temperature shaking at 500 rpm for 60 minutes.
5. **Washing:** Discard the liquid in each well, and fill the wells with Wash Solution (250 µL/well). Stand for 30 seconds and discard the liquid in each well. Repeat the procedure for 4 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. **Washing:** Repeat step 5.
8. **Addition of Streptavidin-HRP solution:** Add 100 µL of enzyme conjugate working buffer to each well.
9. **Second Incubation:** Seal the microplate with microplate sealer and incubate at room temperature shaking at 500 rpm for 30 minutes.
10. **Washing:** Repeat step 5.
11. **Colour development:** Add 100 µL of chromogenic solution to each well, gently shake to mix well, seal the plate with a plate sealer, and place the plate at room temperature for 30 minutes for color development reaction.
12. **Reaction termination**: Add 100 µL of stop solution to each well and immediately perform the detection.

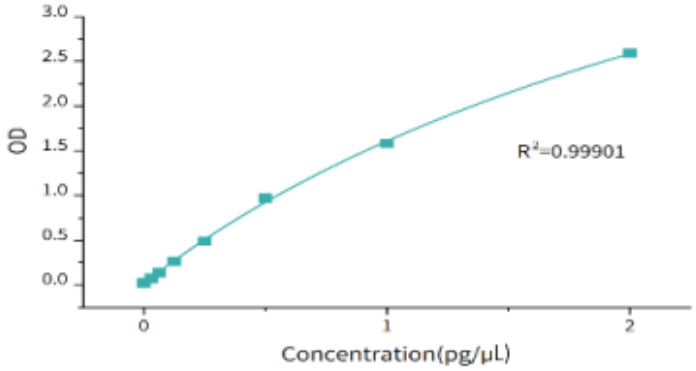
13. **Signal Reading**: Measure the OD values at 450 nm and 630 nm with a plate reader. The measurement should be completed within 30 minutes after reaction termination.

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/µL)	OD value (1)	OD value (2)	Mean value
2	2.8412	2.7362	2.7887
1	1.8725	1.9135	1.8930
0.5	1.0863	1.1207	1.1035
0.25	0.623	0.6055	0.6143
0.125	0.3388	0.3292	0.3340
0.0625	0.1947	0.1885	0.1916
0.0312	0.1192	0.1247	0.1220
0	0.0567	0.0518	0.0543

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.

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.