A005-EN.01

Pyrophosphatase ELISA Kit

Catalog Number：RES-A005

Pack Size: 96 tests

IMPORTANT: Please carefully read this manual before performing your experiment.

For Research Use Only. Not For Use in Diagnostic or Therapeutic Procedures

INTENDED USE

The kit is developed for the detection of Pyrophosphatase in mRNA drug products or semi-manufactures.

It is intended for research use only (RUO).

BACKGROUND

Pyrophosphatase catalyzes the hydrolysis of inorganic pyrophosphate to form orthophosphate, it can hydrolyze inorganic pyrophosphate generated with the reaction to avoid its inhibition of the reaction system. Pyrophosphatase is usually used to increase RNA yield in reverse transcription reactions. As a key raw material for in vitro transcription (IVT) of RNA, Pyrophosphatase needs to detect the residues as a protein component. This ELISA kit can be used to detect the residue of Pyrophosphatase in mRNA stock solution.

In order to support the development of mRNA drugs, ACROBiosystems independently developed Pyrophosphatase ELISA residue detection kit after rigorous methodology verification, which can be used to quantitatively detect the residual content of Pyrophosphatase in mRNA drugs. The quality of mRNA drugs was evaluated during drug development and CMC quality control.

PRINCIPLE OF THE ASSAY

This assay kit is used to measure the levels of Pyrophosphatase by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti- Pyrophosphatase Antibody. Firstly, add the standard samples provided in kit and your samples to the plate, incubate and wash the wells. Then add the Biotin-Anti- Pyrophosphatase Antibody to the plate and form Antibody-antigen-biotinylated antibody complex, incubate and wash the wells. Next add Streptavidin-HRP to the plate, incubate and wash the wells. At last, load the substrate into the wells and monitor solution color from blue to yellow. The reaction is stopped by the addition of a stop solution and the intensity of the absorbance can be measured at 450nm and 630nm. The OD Value reflects the amount of Pyrophosphatase bound.

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PRECAUTIONS

1. This kit is for research use only and is not for use in diagnostic or therapeutic applications.
2. The kit is suitable for cell supernatant, serum and plasma samples.
3. Do not use reagents past their expiration date.
4. Do not mix or substitute reagents with those from other kits or other lot number kits.
5. If samples generate values higher than the highest standard, dilute the samples with the appropriate calibrator diluent and repeat the assay. If cell supernatant samples need step dilution, except for the final dilution with diluent, other intermediate dilutions can be in cell culture medium.
6. Differences in test results can be caused by a variety of factors, including laboratory operator, pipette usage, plate washing technique, reaction time or temperature, and kit storage.
7. This kit is designed to remove or reduce some endogenous interference factors in biological samples, and not all possible influencing factors have been removed.

MATERIALS PROVIDED

Table1. Materials provided

Catalog	Components	Size (96 tests)	Format	Storage	
				Unopened	Opened
RES005-C01	Pre-coated Anti-Pyrophosphatase Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES005-C02	Pyrophosphatase Standard	100 µL	Liquid	2-8°C	2-8°C
RES005-C03	Biotin-Anti-Pyrophosphatase Antibody	15 µg	Powder	2-8°C	-70°C
RES005-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES005-C05	20xWashing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES005-C06	2xDilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES005-C07	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light

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RES005-C08	Stop Solution	7 mL	Liquid	2-8℃	2-8℃

STORAGE

1. Unopened kit should be stored at 2℃-8℃ upon receiving.
2. Find the expiration date on the outside packaging and do not use reagents past their expiration date.
3. The opened kit should be stored per TABLE 1. The shelf life is 30 days from the date of opening.

Note: *a. Do not use reagents past their expiration date.*

b. Find the expiration date on the outside packaging.

REAGENTS/EQUIPMENT NEEDED BUT NOT SUPPLIED

Single or multi-channel micropipettes and pipette tips: need to meet 10 μL, 300 μL, 1000 μL injection requirements;

37ºC Incubator;

Single or dual wavelength microplate reader with 450nm and 630nm filter;

Tubes: 1.5mL,10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

1. Bring all reagents and samples to room temperature (20℃-25℃) before use. If crystals have formed in buffer solution, place the sample in an 37℃ incubator until the crystals have completely dissolved and bring the solution back to room temperature before use.

2. Reconstitute the provided lyophilized materials to stock solutions with distilled, sterile water as recommended in Table 2 and place the materials for 15 to 30 minutes at room temperature with occasional gentle mixing. Avoid vigorous shaking. The reconstituted stock solutions should be stored at -70℃. It is recommended not to freeze-thaw more than 1 times, the packing specification shall not be less than 5 μg.

TABLE 2. RECONSTITUTION METHODS FOR 96 TESTS

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ID	Components	Size	Stock Solution Con.	Reconstitution Buffer and Vol.
RES005-C03	Biotin-Anti-Pyrophosphatase Antibody	15 μg	100 μg/mL	150 μL water

RECOMMENDED SAMPLE PREPARATION

1. Working Solution Preparation

1.1 Preparation of 1×Washing Buffer:

Dilute 50 mL 20×Washing Buffer with ultrapure water/deionized water to 1000 mL.

1.2 Preparation of 1×Dilution Buffer:

Dilute 50 mL 2×Dilution Buffer with 1×Washing Buffer to 100 mL.

1.3 Preparation of Biotin-Anti-Pyrophosphatase Antibody working fluid:

Dilute Biotin-Anti-Pyrophosphatase Antibody reconstituted storage solution to 0.25 μg/mL with 1×Dilution Buffer. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with 1×Dilution Buffer. The prepared working fluid should avoid light. Please prepare it for one-time use only.

2. Preparation of Standard curve

The concentration of the Pyrophosphatase Standard (RES005-C02) is 200 μg/mL, prepare (Std.-0) by diluting 10 μL the Pyrophosphatase Standard into 990 μL Sample Dilution Buffer, mix gently well. Then prepare Std.-1' by diluting 10 μL Std.-0 into 990 μL Sample Dilution Buffer. Finally prepare Std.- 1 by diluting 10 μL Std.-1' into 657 μL Sample Dilution Buffer. As a prepare the highest concentration of standard curve, **Std.-1 (300 pg/mL)**. Prepare 1:1 serial dilution for the standard curve as follows: Pipette 300 μL of Sample Dilution Buffer into each tube. Make sure to mix well every time. Sample Dilution Buffer serves as blank.

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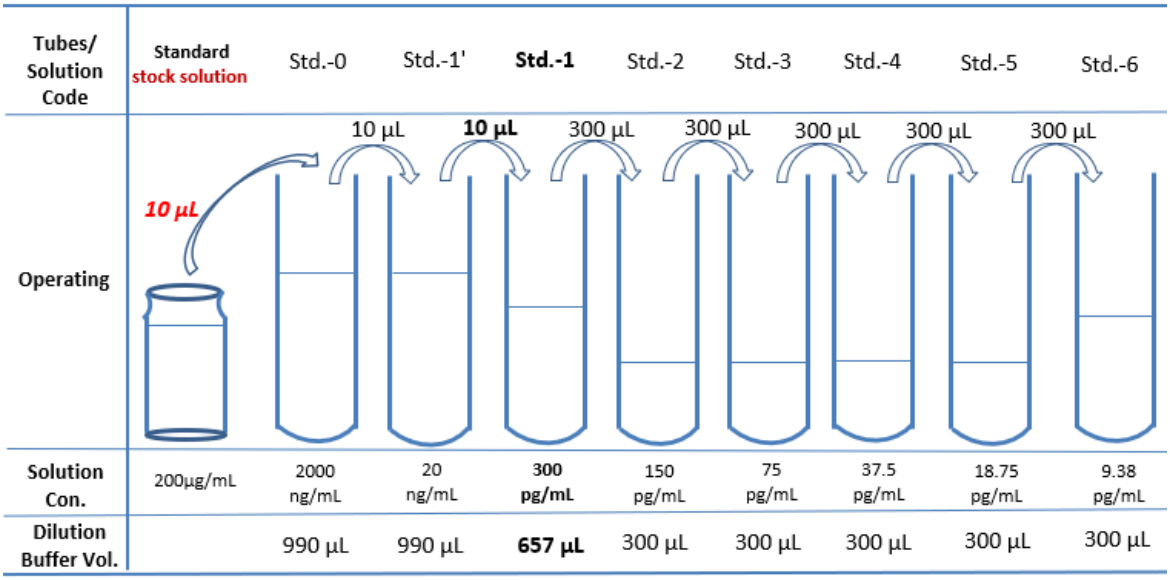

3. Add Samples

Add 100 µL Calibrator and samples to each well. For blank Control wells, please add 100 µL 1xDilution Buffer.

***Note**: It is recommended to set double holes for samples and standard curves to be tested.*

4. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

5. Washing

Remove the remaining solution by aspiration, add 300 µL of 1×Washing Buffer to each well, soak for 10s, remove any remaining 1×Washing Buffer: by aspirating or decanting, invert the plate and blot it against paper towels. Repeat the wash step above for three times.

6. Add Biotin-Anti-Pyrophosphatase Antibody

For all wells, add 100 µL Biotin-Anti-Pyrophosphatase Antibody (dilute to 0.25 µg/mL) working solution. Please prepare it for one-time use only.

7. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

8. Washing

Repeat step 5.

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9. Add Streptavidin-HRP

For all wells, add 100 µL Streptavidin-HRP (dilute at 1:2000) working solution. Please prepare it for one-time use only, avoid light.

10. Incubation

Seal the plate with microplate sealing film and incubate at room temperature for 1 hour.

11. Washing

Repeat step 5.

12. Substrate Reaction

Add 100 µL Substrate Solution to each well. Seal the plate with microplate sealing film and incubate at room temperature for 20 min, avoid light.

13. Termination

Add 50 µL Stop Solution to each well, and tap the plate gently to allow thorough mixing.

Note: *The color in the wells should change from blue to yellow.*

14. Data Recording

Read the absorbance at 450 nm and 630 nm using UV/Vis microplate spectrophotometer within 5 minutes.

Note*: To reduce the background noise, subtract the value read at* OD_{450nm} *with the value read at* $OD_{630\ nm}$.

CALCULATION OF RESULTS

1. Calculate the mean absorbance for each standard, control and sample and subtract average zero standard optical density (O.D.).

2. The standard curve is plotted with the standard concentration as x-axis and the calibrated absorbance value as y-axis. Four parameters logistic are used to draw the standard curve and calculate the sample concentration.

3. Normal range of Standard curve: $R2 \geq 0.9900$.

4. Detection range: 9.38 pg/mL-300 pg/mL. If the OD value of the sample to be tested is higher than 300 pg/mL, the sample shall be diluted with dilution buffer and assay repeated. If the OD value of the sample to be tested is lower than 9.38 pg/mL, the sample should be reported.

QUICK GUILD

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1 **Working fluid perparation and preparation of Standard curve**

↓

2 **Add 100µl standard, sample and blanks to wells**

↓ 18-25℃ 1.0 hour

Aspirate wells & Wash×3

3 **Add 100µl Biotin-Anti- Pyrophosphatase Antibody working solution**

↓ 18-25℃ 1.0 hour

Aspirate wells & Wash×3

4 **Add 100µl Streptavidin-HRP working solution**

↓ 18-25℃ 1.0 hour

Aspirate wells & Wash×3

5 **Add 100µl of Substrate Solution**

↓ 18-25℃ 20 min

Avoid light

6 **Add 50µl Stop Solution and a colorimetric change occurs**

↓ <5 min

7 **Read absorbance**

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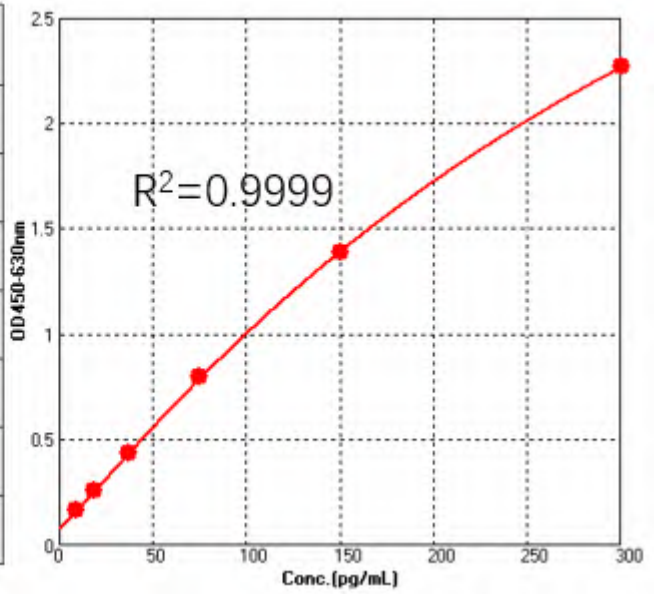
TYPICAL DATA

For each experiment, a standard curve needs to be set for each micro-plate, and the specific OD value may vary depending on different laboratories, testers, or equipments. The following example data is for reference only.

Standard(pg/mL)	OD450-630nm
300	2.268
150	1.392
75	0.799
37.5	0.436
18.75	0.256
9.38	0.167
0	0.071



SENSITIVITY

The minimum detectable concentration of Pyrophosphatase is 1.92 pg/mL. The minimum detectable concentration was determined by adding twice standard deviations to the mean optical density value of twenty zero standard replicates and calculating the corresponding concentration.

PRECISION

1. Intra-assay Precision

Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

2. Inter-assay Precision

Three samples of known concentration were tested in three separate assays to assess inter-assay precision.

	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3

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n	20	20	20	3	3	3
Mean (pg/mL)	256.617	66.092	10.434	251.710	64.829	10.044
SD	14.745	3.085	0.785	5.921	3.090	0.635
CV (%)	5.7%	4.7%	7.5%	2.4%	4.8%	6.3%

***Note**: The example data is for reference only.*

RECOVERY

Three Pyrophosphatase with different concentrations were tested to calculate the recovery rate.

Sample(n=3)	Detect Conc.(pg/mL)	Average Detect Conc. (pg/mL)	Average % Recovery	Range %
High	264.546	260.3	104.1	98.3-110.8
	245.664			
	258.914			
	277.036			
	246.825			
	269.024			
Middle	66.709	64.0	106.7	96.7-112.7
	58.041			
	67.631			
	62.240			
	65.444			
	64.068			
Low	9.725	9.5	95.4	85.2-103.3
	8.520			
	10.329			
	9.022			
	9.524			
	10.128			

PLATE LAYOUT

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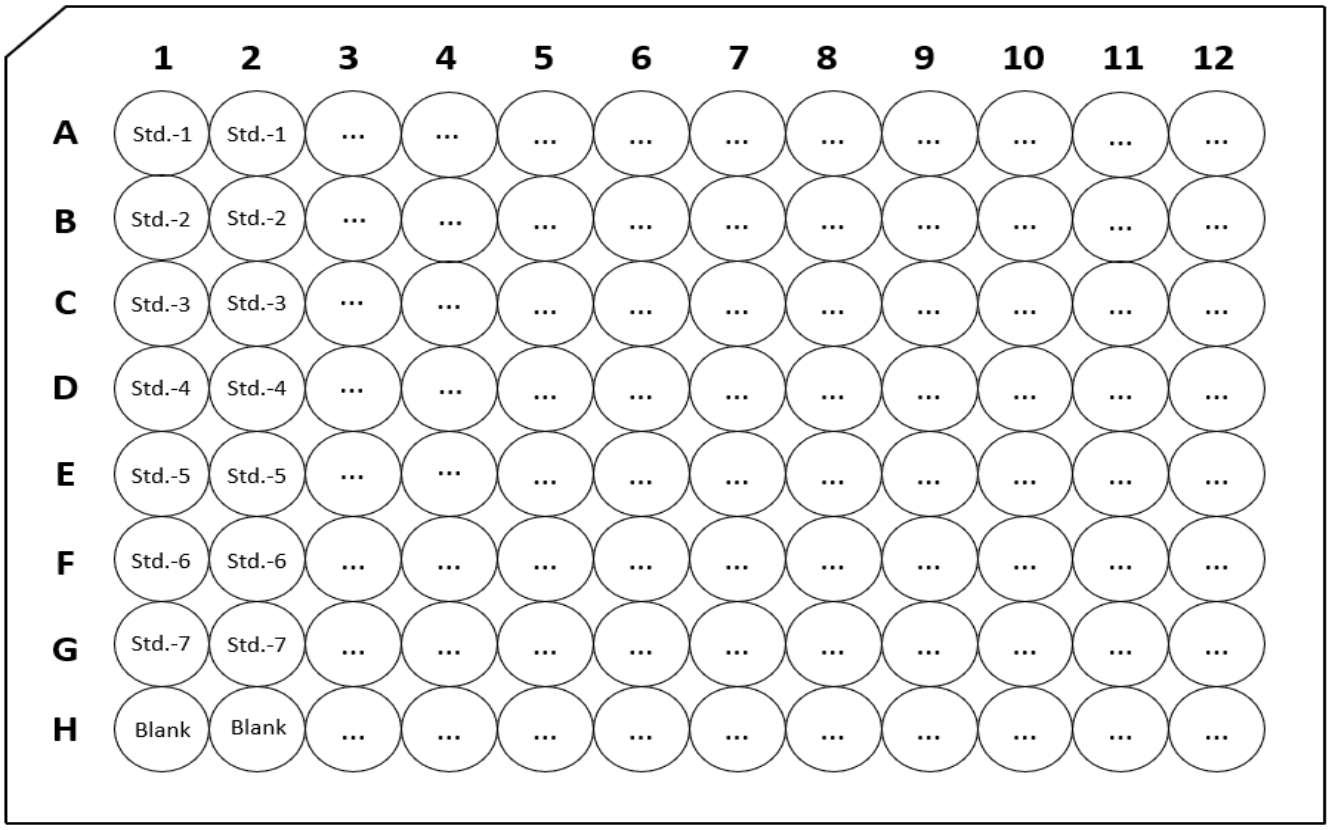

Note: Blank is a Blank Dilution Buffer hole.

TROUBLESHOOTING GUIDE

Problem	Cause	Solution
Poor standard curve	* Inaccurate pipetting	* Check pipettes
Large CV	* Inaccurate pipetting * Air bubbles in wells	* Check pipettes * Remove bubbles in wells
High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time

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Samples are reading too high, but standard curve looks fine	* Samples contain cytokine levels above assay range	* Dilute samples and run again
Drift	* Interrupted assay set-up * Reagents not at room temperature	* Assay set-up should be continuous - have all standards and samples prepared appropriately before commencement of the assay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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