RES18-EN.01

T7 RNA polymerase ELISA Kit

Catalog Number：RES-A018

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 Procedure

INTENDED USE

The kit is developed for the detection of T7 RNA polymerase in mRNA drug products or semi-manufactures.

It is intended for research use only (RUO).

BACKGROUND

T7 RNA Polymerase is a DNA-dependent RNA polymerase with strict specificity for the T7 phage promoter. The enzyme is widely used for the synthesis of specific transcripts from DNA in the 5'→3' direction. It is also a key raw material for in vitro transcription (IVT) of RNA. T7 RNA Polymerase needs to be detected the residues as a protein component when it's used in IVT. This ELISA kit can be used to detect the residue of T7 RNA Polymerase in mRNA stock solution.

In order to support the development of mRNA drugs, ACROBiosystems independently developed T7 RNA Polymerase ELISA residue detection kit after rigorous methodology verification, which can be used to quantitatively detect the residual content of T7 RNA Polymerase 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 T7 RNA Polymerase by employing a standard sandwich-ELISA format. The micro-plate in the kit has been pre-coated with Anti-T7 RNA polymerase 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-T7 RNA polymerase 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 450 nm and 630 nm. The OD Value reflects the amount of T7 RNA Polymerase 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 the Standard and Sample Dilution Buffer (5×) does not clarify, please centrifuge and use.
6. 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.
7. 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.
8. 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
RES018-C01	Pre-coated Anti-T7 RNA polymerase Antibody Microplate	1 plate	Solid	2-8°C	2-8°C
RES018-C02	T7 RNA polymerase Standard	100 µL	Liquid	2-8°C	2-8°C
RES018-C03	Biotin-Anti-T7 RNA polymerase Antibody	150 µL	Liquid	2-8°C	2-8°C
RES018-C04	Streptavidin-HRP	50 µL	Liquid	2-8°C, avoid light	2-8°C, avoid light
RES018-C05	20×Washing Buffer	50 mL	Liquid	2-8°C	2-8°C
RES018-C06	Biotin-Antibody and Streptavidin-HRP Dilution Buffer	50 mL	Liquid	2-8°C	2-8°C
RES018-C07	Standard and Sample Dilution Buffer (5×)	30 mL	Liquid	2-8°C	2-8°C
RES018-C08	Substrate Solution	12 mL	Liquid	2-8°C, avoid light	2-8°C, avoid light

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RES018-C09	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 450 nm and 630 nm filter;

Tubes: 1.5mL, 10mL;

Timer;

Reagent bottle;

Deionized or distilled water.

REAGENT PREPARATION

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.

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×Standard and Sample Dilution Buffer:

Dilute 30 mL 5×Standard and Sample Dilution Buffer with 1×Washing Buffer to 150 mL.

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1.3 Preparation of Biotin-Anti-T7 RNA polymerase Antibody working fluid:

Dilute Biotin-Anti-T7 RNA polymerase Antibody reconstituted storage solution with Biotin-Antibody and Streptavidin-HRP Dilution Buffer at 1:200. Please prepare it for one-time use only.

1.4 Preparation of Streptavidin-HRP working fluid:

Dilute Streptavidin-HRP at 1:2000 with Biotin-Antibody and Streptavidin-HRP 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 T7 RNA polymerase Standard (RES018-C02) is 200 μg/mL, prepare (Std.-0) by diluting 5 μL the T7 RNA polymerase Standard into 495 μL Sample Dilution Buffer, mix gently well. Then prepare Std.- 1 by diluting 15 μL Std.-0 into 585 μL Sample Dilution Buffer. As a prepare the highest concentration of standard curve, **Std.-1 (50 ng/mL)**. Prepare 1:1 serial dilutions 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.

Tubes/ Solution Code	Standard stock solution	Std.-0	**Std.-1**	Std.-2	Std.-3	Std.-4	Std.-5	Std.-6	Std.-7
Operating	5 μL	**15 μL**	300 μL	300 μL	300 μL	300 μL	300 μL	300 μL	
Solution Con.	200μg/mL	2000 ng/mL	50 ng/mL	25 ng/mL	12.5 ng/mL	6.25 ng/mL	3.13 ng/mL	1.56 ng/mL	0.78 ng/mL
Dilution Buffer Vol.		495 μL	**585 μL**	300 μL	300 μL	300 μL	300 μL	300 μL	300 μL

3. Add Samples

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

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Standard and Sample Dilution Buffer.

***Note**: It is recommended to set doeble 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 10 s, 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-T7 RNA polymerase Antibody

For all wells, add 100 µL Biotin-Anti-T7 RNA polymerase Antibody (dilute at 1:200) 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.

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.

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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 (OD).
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: $R^2 \geqslant 0.9900$.
4. Detection range: 0.78 ng/mL-50 ng/mL. If the OD value of the sample to be tested is higher than 50 ng/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 0.78 ng/mL, the sample should be reported.

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QUICK GUILD

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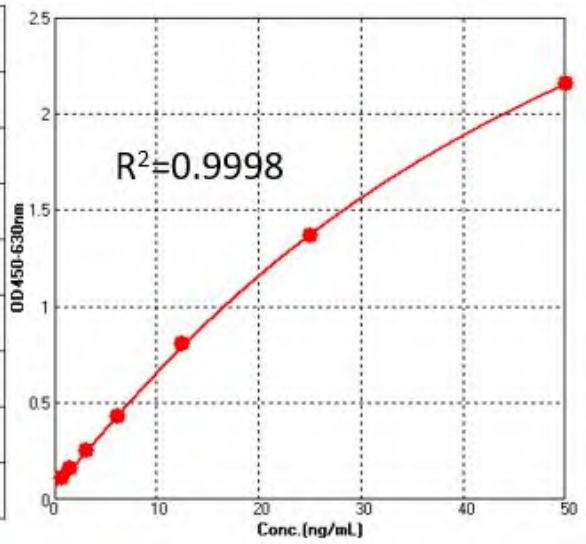
TYPICAL DATA

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.

T7 RNA polymerase Standard(ng/mL)	OD450-630nm
50	2.156
25	1.370
12.5	0.809
6.25	0.428
3.125	0.255
1.5625	0.159
0.78125	0.107
0	0.062



SENSITIVITY

The minimum detectable concentration of T7 RNA polymerase is 0.161 ng/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.

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	Intra-assay Precision			Inter-assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	3	3	3
Mean (ng/mL)	36.606	12.845	1.779	37.457	13.009	1.801
SD	2.255	0.383	0.149	0.805	0.213	0.023
CV (%)	6.2%	3.0%	8.4%	2.1%	1.6%	1.3%

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

RECOVERY

Three T7 RNA polymerases with different concentrations were tested to calculate the recovery rate.

Sample(n=3)	Detect Conc.(ng/mL)	Average Detect Conc. (ng/mL)	Average % Recovery	Range %
High	33.606	35.311	88.3	82.9-93.6
	35.925			
	37.432			
	36.014			
	35.746			
	33.146			
Middle	9.204	9.288	92.9	86.5-100.2
	10.024			
	8.867			
	9.482			
	9.502			
	8.650			
Low	1.732	1.867	93.3	81.4-107.7
	2.101			
	1.627			
	2.153			
	1.855			
	1.732			

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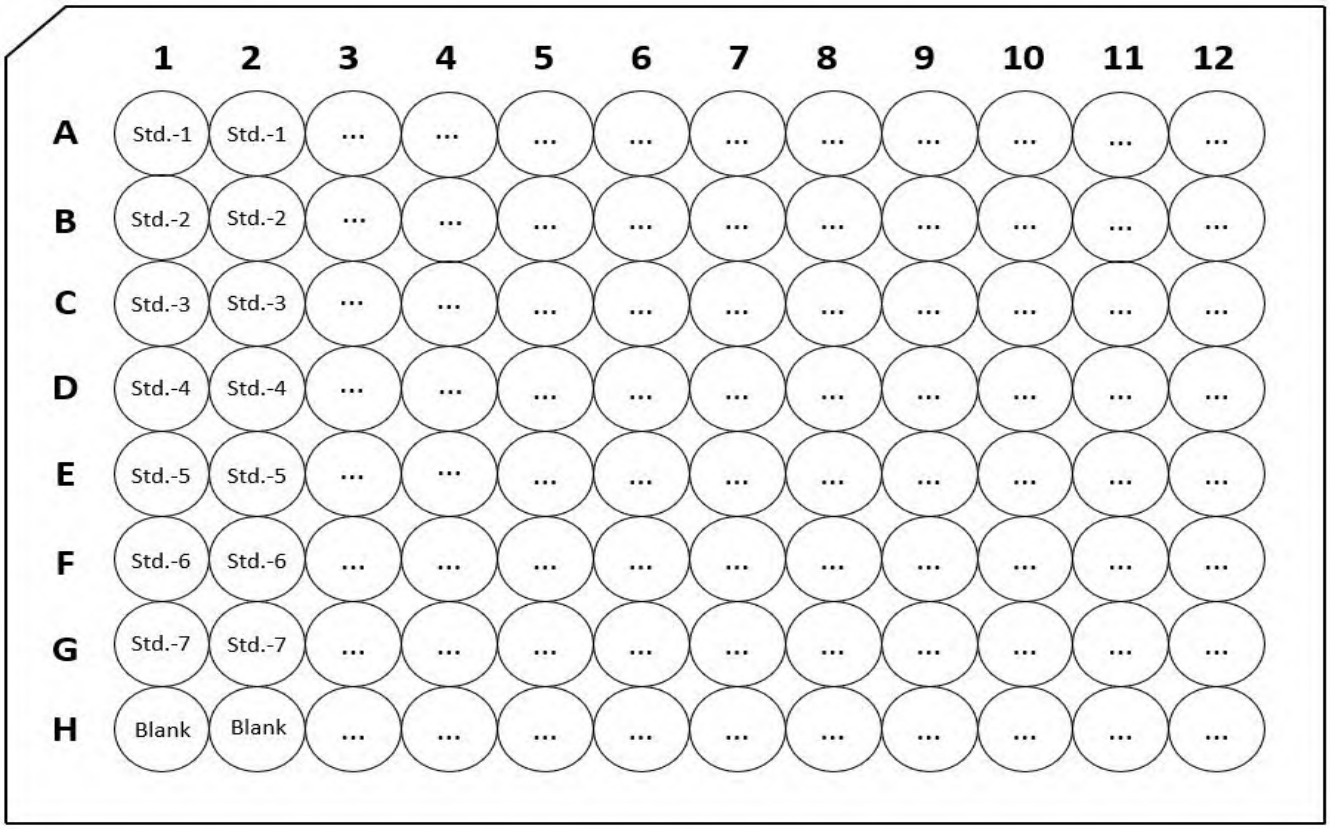
PLATE LAYOUT



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

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High background	* Plate is insufficiently washed * Contaminated wash buffer	* Review the 11manual for proper wash. * Make fresh wash buffer
Very low readings across the plate	* Incorrect wavelengths * Insufficient development time	* Check filters/reader * Increase development time
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 theassay * Ensure that all reagents are at room temperature before pipetting into the wells unless otherwise instructed in the antibody inserts

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