LEADGENE
LEADGENE BIOMEDICAL, INC.

Product Information & Manual

Information of other products is available at: www.leadgenebio.com

T7 RNA Polymerase ELISA Kit

Enzyme-linked immunosorbent assay for quantitative detection of T7 RNA Polymerase

Catalogue Number LDG00024E

For Research Use Only. Not for use in diagnostic and therapeutic procedures.

RUO

96 wells

Store at 2-8℃

LEADGENE BIOMEDICAL, INC.
No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan
TEL: +886-6-2536677 • FAX: +886-6-2531536
Email: info@leadgene.com.tw • www.leadgenebio.com

Leadgene® T7 RNA Polymerase ELISA Kit

1. Introduction

Bacteriophage T7 RNA Polymerase is a DNA-dependent RNA polymerase with high specificity for the T7 promoter. This enzyme catalyzes the 5′ →3′ synthesis of RNA from DNA downstream from its promoter.

Leadgene® T7 RNA Polymerase ELISA kit is an enzyme-linked immunosorbent assay (ELISA) for the quantitative detection of T7 RNA Polymerase level in sample solution. The T7 RNA Polymerase ELISA kit is for research use only (RUO). Not suitable for use in diagnostic or therapeutic procedures.

2. Test principle

T7 RNA Polymerase ELISA kit is used to detect T7 RNA Polymerase in samples by sandwich ELISA method. This assay uses microplate pre-coated with mouse anti-T7 RNA Polymerase monoclonal antibody to the solid phase. T7 RNA Polymerase in the samples conjugates on solid phase and then react with the HRP conjugated mouse anti-T7 RNA Polymerase monoclonal antibody. Subsequent wash steps will residual unbound antibody. After incubation with substrate solution, the reaction is determined by the absorbance at 450 nm. Quantification of T7 RNA Polymerase protein level is accomplished by comparing the absorbance with standard curve.

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

3. Reagents provided and reconstitution

Reagents (Store at 2-8℃)	Quantity 1x96 well kit	Reconstitution
T7 RNA Polymerase ELISA plate Stripwell microplate with 96 anti-T7 RNA Polymerase monoclonal antibodies coated wells	96 wells (12 x 8-well strips)	**Ready** for use
Standard T7 RNA Polymerase lyophilized from buffered protein solution with preservatives	2 vials (Lyophilized form)	**Refer to the vial label for reconstitution volume.** Reconstitute by adding **Standard reconstitution buffer** to be a stock solution of 100 ng/mL. (see procedure, section 8.(2))
Standard reconstitution buffer Buffered protein solution with preservatives	2 vials (1.1 mL)	**Ready** for use
Standard & Sample diluent buffer Buffered protein solution with preservatives	1 vial (12 mL)	**Ready** for use
HRP-antibody conjugate HRP conjugated anti-T7 RNA Polymerase monoclonal antibody in buffered protein solution with preservatives	1 vial (70 µL)	**Dilute** 200 x with **HRP-antibody conjugated diluent buffer** (see reagent preparation, section 5.A)
HRP-antibody conjugated diluent buffer Buffered solution with preservatives	1 vial (12 mL)	**Ready** for use
20 X wash buffer 20-fold concentrated solution of buffered surfactant with preservatives	1 vial (15 mL)	**Dilute** 20 x with distilled water (see reagent preparation, section 5.B)
TMB Chromogenic substrate (tetramethylbenzidine) for HRP	1 vial (12 mL)	**Ready** for use
Stop solution H_2SO_4 solution	1 vial (6 mL)	**Ready** for use
Microplate sealing film	1 sheet	N/A

4. Materials required but not provided

(1) High quality distilled water

(2) 10 mL graduated pipettes

(3) 5 µL to 1000 µL adjustable single-channel micropipettes with disposable tips

(4) 50 µL to 300 µL adjustable multi-channel micropipettes with disposable tips

(5) Multi-channel micropipette reservoir

LEADGENE www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

(6) Disposable microcentrifuge tubes
(7) Beakers, flasks, cylinders necessary for preparation of reagents
(8) Timer
(9) Magnetic stirrer
(10) Vortex mixer
(11) Washer for microplates
(12) Incubator capable of maintaining temperature at 37±1°C
(13) Stripwell microplate spectrophotometer capable of reading at 450 nm
(14) Clean paper towels
(15) Disposable gloves
(16) Discard container for bio-medical waste

5. Reagent preparation

All the working reagents should be prepared with adequate volume and discarded at the end of the day.

A. **Working HRP-antibody conjugate (1X):** Dilute 1 volume of **HRP-antibody conjugate** with 199 volumes of **HRP-antibody conjugated diluent buffer** and homogenize by vortex.
B. **Working wash buffer (1X):** Dilute 1 volume of **20 X wash buffer** with 19 volumes of distilled water and homogenize by using a magnetic stirrer.

6. Storage and expiration date of reagents

- Before opened or reconstituted, all kit reagents should be kept properly at 2-8℃. Please see the box front label for expiration date.
- Once opened, the kit should be used within 2 weeks, and the remaining reagents should be immediately returned to 2-8℃ after used, except the reconstituted standard, it must be stored at -80℃.
- Avoid multiple freeze-thaw cycles of the frozen reconstituted standard, and if stored properly at -80℃, it should be valid for maximum 2 weeks.
- Unused strips must be stored at 2-8℃ in a sealed bag containing a desiccant and should be used as soon as possible.
- All working reagents, Working HRP-antibody conjugate (1 X) and Working wash buffer (1 X), should be prepared freshly and used on the same day.
- Alterations in physical appearance of kit components may indicate instability or deterioration.

7. Precautions & warnings

In order to obtain reproducible test results, the following rules should be strictly obeyed:

- All reagents and specimens should be considered as potentially hazardous. We therefore recommend that this product is handled by those persons who have been properly trained.
- Wear suitable protective clothing and disposable gloves.
- Care should be taken to avoid reagents (especially TMB and Stop solution, which contains H_2SO_4) contacting with skin

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

or eyes. If contacted, wash immediately and thoroughly with plenty of clean water.

- This product is intended for *Research use only* and is not for use in diagnostic and therapeutic procedures.
- This product is designed for a single, one-time use only.
- The assay should be performed as outlined in this manual, and in accordance with all instructions.
- Do not use expired or damaged products.
- Do not mix or substitute reagents with those from different lots or other sources.
- Bring all the reagents and specimens to 15-30℃ prior to use.
- Thoroughly and gently mix all the reagents and specimens prior to use.
- Do not expose all the reagents to strong light during storage or incubation.
- Avoid contact of TMB with metal to prevent color development. The color of TMB should be colorless. If a blue color develops before use, indicating it is unusable, it must be discarded.
- Use disposable graduated pipettes and tips to avoid microbial contamination or cross-contamination of reagents or specimens which may invalidate the test.
- After use, all the reagents and specimens should be regarded as medical waste with risk of biological infection and properly disposed of in accordance with national regulations.

8. Procedure

(1) Evaluate the number of stripwell required to test the samples. Put the stripwells at room temperature (15-30℃) before use. The unused strips should be resealed in the bag and stored at 2-8℃. Each standard, blank, and sample should be assayed in duplicate.

(2) Standard and sample preparation:

- **Standard preparation (in microcentrifuge tubes):**

- **Refer to the vial label for reconstitution volume.** Reconstitute the lyophilized standard with Standard reconstitution buffer to the concentration of 100 ng/mL. Vortex for 1 min and incubate for at least 10 minutes. Aliquot and store the standards at -20°C.
- Add 450 µL Standard & Sample diluent buffer to 50 µL of 100 ng/mL standard to make a 10 ng/mL standard (Tube 1).
- Adding 250 µL of Standard & Sample diluent buffer to 250 µL of 10 ng/mL standard to make a 5 ng/mL standard (Tube 2).
- Repeat the above procedure to make serial diluted standards (Tube 3-7).
- Tube 8 is blank which only containing Standard & Sample diluent buffer.

- **Sample preparation:**

- 100 µL Sample. If the initial assay found samples contain T7 RNA Polymerase higher than the highest standard, the samples can be diluted with Standard & Sample diluent buffer and then re-assay the samples.

(3) Add 100 µL of standards, blanks or samples into T7 RNA Polymerase ELISA stripwell microplates (see Table 1), then add 100 µL of Working HRP-antibody conjugate into each well. Cover with microplate sealing film and incubate sealed plate at 37℃ for 1 hour in the dark.

(4) Remove the sealing film, aspirate the liquid from each well and then wash the plate six times with 300 µL Working wash

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

buffer per well. After the last wash, tap stripwells on clean absorbent paper to remove excess wash buffer.

(5) Add 100 µL of TMB into each well. Incubate for 10 minutes at 37℃ in the dark.

(6) Add 50 µL Stop solution into each well.

(7) Read the absorbencies immediately at 450 nm after the Stop solution is added.

Table 1 An example of orientation of standards, blanks and samples in the stripwells microplate

	1	2	3	4
A	Standard 1 (10 ng/mL)	Standard 1 (10 ng/mL)	Sample 1	Sample 5
B	Standard 2 (50 ng/mL)	Standard 2 (5 ng/mL)	Sample 1	Sample 5
C	Standard 3 (2.5 ng/mL)	Standard 3 (2.5 ng/mL)	Sample 2	Sample 6
D	Standard 4 (1.25 ng/mL)	Standard 4 (1.25 ng/mL)	Sample 2	Sample 6
E	Standard 5 (0.625 ng/mL)	Standard 5 (0.625 ng/mL)	Sample 3	Sample 7
F	Standard 6 (0.3125 ng/mL)	Standard 6 (0.3125 ng/mL)	Sample 3	Sample 7
G	Standard 7 (0.15625 ng/mL)	Standard 7 (0.15625 ng/mL)	Sample 4	Sample 8
H	Blank	Blank	Sample 4	Sample 8

9. Internal quality control

- The average absorbance of Blank: ≦ 0.1
- The average absorbance of highest concentration of standard (10 ng/mL): ≧ 1.8

10. Calculation of results

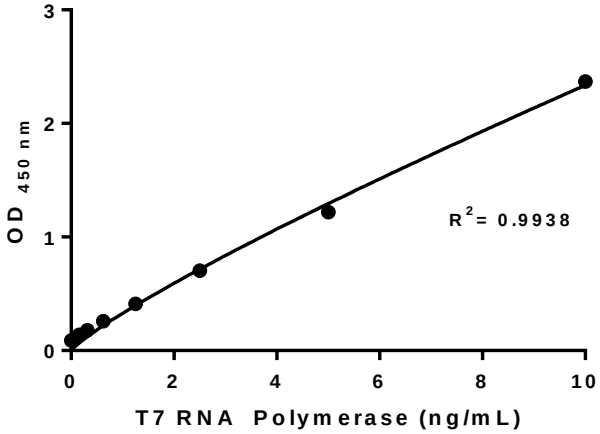
- The standard curve is generated by plotting the average absorbance of standards (linear, y-axis) against the corresponding standard concentrations (linear, x-axis) using linear curve fit.
- The T7 RNA Polymerase concentrations of samples are determined by interpolation on the calibration curve.
- If the assay concentrations of samples are higher than 10 ng/mL, the samples should be diluted with Standard & Sample diluent buffer and re-assay again.

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

Typical data

The following data are for demonstration only

Standard	T7 RNA Polymerase concentration (ng/mL)	OD 450 nm	
1	10	2.411	2.328
2	5	1.23	1.209
3	2.5	0.701	0.705
4	1.25	0.413	0.409
5	0.625	0.26	0.256
6	0.3125	0.184	0.175
7	0.15625	0.143	0.132
Blank	0	0.09	0.088



11. Assay limitations

- Sample should be centrifuged to remove debris.

12. Performance characteristics

Sensitivity

- The limit of detection (LoD) of T7 RNA Polymerase ELISA kit is 0.037 ng/mL.
- The limit of quantification (LoQ) of T7 RNA Polymerase ELISA kit is 0.124 ng/mL.

LEADGENE
www.leadgenebio.com
TEL: +886-6-253-6677 • Email: info@leadgene.com.tw • No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan

LEADGENE BIOMEDICAL, INC.
No.9, Ln. 147, Zhengbei 1st Rd., Yongkang Dist., Tainan City 710, Taiwan R.O.C.
TEL: +886-6-2536677
FAX: +886-6-2531536
Email: info@leadgene.com.tw
www.leadgenebio.com