Phage Titration ELISA

Enzyme Immunoassay for the Quantitative Determination of Purified M13 Bacteriophage Particles.

Art. No.: **PRPHAGE**

Contents: **12 × 8 Determinations**

Storage: **2 - 8°C**

For research use only!

1. Introduction

The phage display technology is a powerful and well-established tool for the investigation of protein-protein interactions and for the generation of human antibodies for in vitro diagnostics and in vivo therapy (Rondot et al. 2001). Generally several rounds of selection on an immobilized antigen are required to enrich and isolate the binding phage. The need to determine phage titers before and after each round to monitor the panning process still represents a major time and material consuming factor within this procedure. Usually, the phages are titered by infecting E. coli with serial dilutions of phage, plating the bacteria on agar plates and counting the colony (cfu) or plaque forming units (pfu) after over-night incubation (Koch et al., 2000). To determine the relative number of phage particles, however, immunotitration by PROGEN's Phage Titration ELISA offers a fast, sensitive and reproducible alternative for titration of M13 bacteriophage preparations.

2. Test Principle

The assay is based on a sandwich ELISA technique. A monoclonal antibody specific for the pVIII protein on the surface of the bacteriophage M13 is coated onto microtiter strips and is used to capture M13 particles from the specimen. Captured M13 particles are detected by a peroxidase conjugated monoclonal antibody to pVIII, which is bound to the immune complex. Addition of substrate solution results in a color reaction which is proportional to the amount of specifically bound phage particles. The absorbance is measured photometrically at 450 nm.

The kit control provided contains a lyophilized M13 particle preparation. It shows a typical titration curve when used in a serial dilution (Fig. 1) and allows the quantitation of samples with an unknown particle titer.

3. Material Required

Test tubes for specimen dilutions
Precision pipettes , sterile pipette tips, distilled water
Incubator for 37°C, ELISA Reader (450 nm)

4. Contents of Test Kit

MTP	Microtiter Plate, 12 × 8-well-strips, coated with mouse monoclonal antibody to M13 in resealable aluminum bag with desiccant. Ready-to-use.
KC	Kit Control (purified M13 particles), lyophilized, 2 vials. Reconstitute before use.
CON 20x	Anti-M13 Peroxidase Conjugate 20x, 0.75 mL, 1 vial. Dilute before use.
ASSB 20x	Assay Buffer 20x, 20 mL, 1 bottle. Dilute before use.
TMB	Substrate, (tetramethylbenzidine), 12 ml, 1 bottle. Ready-to-use.
STOP	Stop Solution, 13 mL, 1 bottle. Ready-to-use.

Adhesive foil for covering ELISA test strips, 1 pc.

Plastic bag for storing unused ELISA test strips, 1 pc.

All components except S and STOP contain a preservative!

5. Preparation of Reagents

Allow kit to reach room temperature (20-26°C, RT). Buffer concentrates may contain salt crystals which dissolve quickly at 37°C. Let buffer reach room temperature (20-26°C) before use.

Store unused strips in the resealable aluminum bag with desiccant at 2-8°C.

Dilute required volumes of reagents immediately before use!

***Preparation of ready-to-use solutions*:**

Assay buffer: Dilute 1:20 with distilled water for ready-to-use Assay buffer.

CON* peroxidase conjugate: Dilute 1:20 with ready-to-use Assay buffer for ready-to-use anti-M13 peroxidase conjugate.

KC Reconstitute with **500 µl** distilled water; contains approx. 1x 10^8 of particles/mL (see label for exact concentration).

* Dilute immediately before use!

6. Stability of Reagents

Store the test kit and components at 2-8°C.The unopened reagents are stable until the expiry date indicated.

Stability after opening:

6 months at 2-8°C:

ASSB 20x, CON 20x, TMB, MTP (in aluminum bag with desiccant)

7. Kit Control and Specimen Dilution

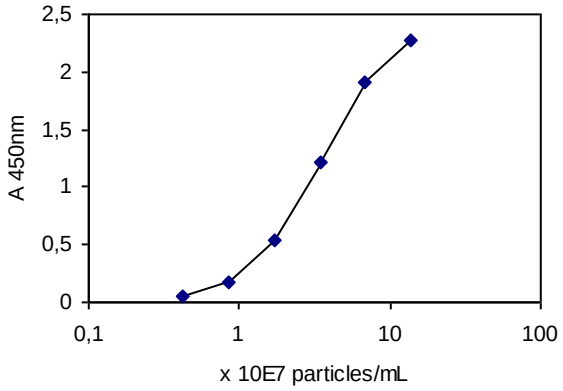
The range of the ELISA covers 5×10^6 – 1×10^8 particles/mL. Dilute specimen containing M13 particles

to reach a concentration within the linear range of the ELISA using ready-to-use Wash buffer.

Dilute **specimen** in steps of e.g. 1:2. A minimum of 2-3 different dilutions should be tested.

Dilute the reconstituted **Kit Control** in ready-to-use Assay buffer.

Fig. 1: Example of a Titration Curve



8. Test Procedure

1. Pipette 100 µL of ready-to-use Assay buffer (Blank), serial dilutions of Kit Control and specimen (both diluted in ready-to-use Assay buffer) into the wells of the microtiter strips. Seal strips with adhesive foil provided and incubate for 1 h at 37°C.
2. Empty contents of microtiter strips.

   Fill wells with 200 µL each of ready-to-use Assay buffer, incubate approximately 5 sec, empty and tap inverted plate onto absorbent paper. Repeat washing step 2×.
3. Pipette 100 µL per well of ready-to-use peroxidase conjugate. Seal strips with adhesive foil and incubate for 1 h at 37°C.
4. Repeat washing step as described in 2.
5. Pipette 100 µL per well of ready-to-use substrate. Incubate for 15 min at RT.
6. Stop color reaction by adding 100 µL of stop solution into each well.
7. Measure intensity of color reaction with a microplate reader at 450 nm wavelength within 30 min.

9. Calculation of Results

Results are calculated from the standard curve by 4-parameter analysis. The standard curve is generated using the Blank subtracted OD-readings (y-axis) of the serial dilution of the Kit Control with the assigned particle titer (x-axis) analogous to Tab. 2 and Fig 1.

4-parameter curve fit: $y = (A–D) / (1 + (x/ C)^B)+ D$

Most computers, plate reader software or calculators are capable to perform this analysis.

Use this standard curve for the calculation of the particle titer of unknown specimens.

10. Quality Control

Kit Control (undiluted)	OD > 1.3
Blank	OD < 0.2

11. Notes for the User

Security notes

Stop solution (sulfuric acid) and components of substrate (TMB) may cause skin irritations. If acid or TMB should come into contact with eyes, rinse out immediately with plenty of water and consult a physician!

All components except TMB and STOP contain a preservative! Do not swallow! Avoid any contact with skin or mucous epithelia!

Safety data sheet is available on request!

Disposal considerations

Product: Chemicals and biological materials must be disposed of in compliance with the respective national regulations.

Packaging: Packaging must be disposed of in compliance with the country-specific regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified differently, non-contaminated packaging may be treated like household waste or recycled.

Measures after damage during transport

If a kit is considerably damaged, please contact the manufacturer or local distributor. Do not use considerably damaged components for a test procedure. Store such components or kits until the complaint is processed.

12. Literature

1. Rondot S, Koch J, Breitling F and Dübel S (2001). A helper phage to improve single-chain antibody presentation in phage display. Nature Biotechnology *19*, 75-8.
2. Koch J, Breitling F and Dübel S (2000). Rapid titration of filamentous bacteriophage (M13) on nitrocellulose membranes. BioTechniques, *29*, 1196-202.

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