SuperBrite™ ELISA HRP Chemiluminescence Substrate Kit

09/18

(Cat # K4005-500, 500 assays, Store at 4°C)

I. Introduction:

Enzyme-linked immunosorbent assay (ELISA) is the most commonly used method for detecting or identifying substances such as peptides, proteins, antibodies and hormones. The sensitivity of ELISA depends on the detection limits, colorimetric detection limits the assay sensitivity for the colorimetric substrates used. Chemiluminescent detection is an alternative variation of the standard enzyme colorimetric detection, characterized by its high sensitivity, broader dynamic range, and high signal-to-noise ratio. In chemiluminescence detection, horse radish peroxidase (HRP) substrates have shown improved sensitivity over colorimetric substrates. BioVision's SuperBrite™ ELISA HRP Chemiluminescence substrate provides excellent sensitivity and has been successfully used in high throughput enzymatic immunoassays to be run on a microplate chemiluminescence reader.

II. Applications:

- Fast and sensitive substrate for detecting HRP activity in ELISA assays.
- Extensive linear detection range.
- Signal development within 1 minute.

III. Kit Contents:

Components	K4005-500	Cap Code	Part Number
Reagent A Reagent B	25 ml 25 ml	NM Amber	K4005-500-1 K4005-500-2

IV. User Supplied Reagents and Equipment:

- Reagents, antibodies and buffers needed for ELISA assays
- 96-well luminescence black plate (high binding) for plate coating
- 96-well plate luminescence reader.

V. Storage Condition:

Store at 4°C, protect from light. Read the entire protocol before performing the assay.

VI. ELISA HRP Chemiluminescence Substrate Kit Protocol:

1. Calculate the total volume of the working solution: 0.1 ml / well × quantity of wells (add reserve 0.1 - 0.2 ml to the total volume). Prepare fresh working solution by mixing Reagent A and B at 1:1 ratio (avoid light). Premixed working solution is stable at room temperature for up to 60 minutes.
2. Carry out standard optimized ELISA protocol including the incubation step with the HRP detection reagent (i.e. HRP-SA or HRP-anti-Ab conjugates).
3. Wash plate 5 times with 300 µl Wash Buffer. During each wash, leave the Wash Buffer in the wells for 1-2 minutes, and then remove all residual liquid from the wells by aspiration.
4. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert and clap the plate on absorbent filter papers or other absorbent materials.
5. Add 100 µl premixed ELISA HRP Chemiluminescence Substrate to each well with a multi-channel pipette.
6. Measure the luminescence signal within 2 minutes.

(A) **(B)**

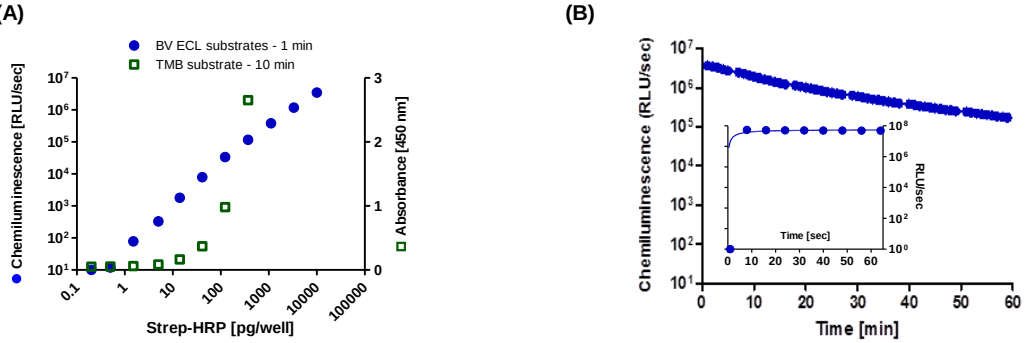

Figure: (A) Sensitivity comparison between ELISA Chemiluminescence Substrate (●) and TMB colorimetric Substrate (□). Chemiluminescence substrate gives wider linear range. (B) Kinetics of signal development. The peroxidase reaction reaches its steady state within 1-2 minutes, the signal gradually degrades as oxidized luminol decays.

VII. Related Products:

- UltraPolymer Goat Anti-Rabbit/Mouse IgG (H&L) HRP Cocktail (A1280)
- UltraPolymer Goat Anti-Rabbit IgG (H&L) HRP (A1274)
- UltraPolymer Goat Anti-Rat IgG (H&L) HRP (A1277)
- UltraPolymer Donkey Anti-Sheep IgG (H&L) HRP (A1279)
- Goat Anti-Rat IgG (H&L) HRP (6908)

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