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SuperBrite™ ECL Western Blot Substrate/Detection Kit

(Store at 4°C)

Cat. No.: K824-50
Contains: 50 ml SuperBrite™ ECL Western Blotting detection solutions, sufficient for 30 mini-gel blots or 2500 cm^2 of membrane

I. Introduction:

BioVision's SuperBrite™ ECL kit includes second-generation ECL substrates. They are highly sensitive, non-radioactive, and enhanced luminol-based chemiluminescent substrates for easy detection of horseradish peroxidase (HRP) on immunoblots. Blots can be repeatedly exposed to X-ray film or a CCD camera to obtain optimal results or stripped off the immune-detection reagents and re-probed.

II. Application:

- Western Blotting, Dot Blotting.

III. Advantages:

- **Minimal volumes required:** Can be used for 30 mini-gel blots or 2500 cm^2 of membrane.
- **Exceptional Sensitivity:** Detects targets in the low picogram levels.
- **Multiple imaging options:** Compatible with X-Ray, CCD and laser based imaging.
- **Ready to Use:** No additional reconstitutions required.
- **Stable signal:** Signals developed are stable.
- **Economical:** Available at the most competitive rates.

IV. Kit Contents:

Components	K824-50	Cap Code	Part Number
Reagent A	25 ml	Clear	K824-50-1
Reagent B	25 ml	Amber	K824-50-2

V. User supplied reagents:

- Western blot membrane
- X-ray film or imaging system

VI. Storage and Handling:

Store at 4°C. Read the entire protocol before performing the assay.

VII. ECL Kit Protocol:

1. Prepare fresh working solution by mixing Reagent A and B at a 1:1 ratio. Use 0.02 ml working solution per cm^2 of membrane.
2. Immediately add the working solution to the blot and incubate for less than 30 sec. at room temperature.
4. Remove the blot from working solution and drain excess reagent.
5. Place the blot in a clear plastic sheet protector or clear plastic wrap. Remove bubbles.
6. Expose the blot to an X-ray film or imaging system.

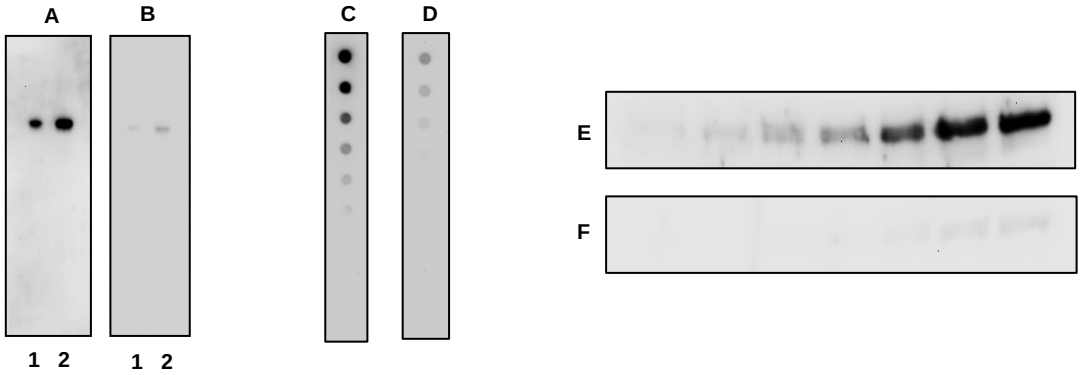

Figure: Comparison of BioVision's second-generation ECL substrate with competitor's ECL substrate: Western Blot of protein Procathepsin K (Cat # 1027) was performed using this kit (**A**) and competitor's kit (**B**). The protein was separated on a 17% SDS-PAGE and transferred to PVDF membrane. The blot was incubated with 4ug/ml anti-rabbit Procathepsin K antibody (Cat. # 6667) overnight at 4°C, followed by incubation with HRP-conjugated goat anti-Rabbit IgG at 1:5,000 dilution for 1 hr at room temperature. The membrane was exposed to G:BOX for 3 min. Lane 1: 1 ng; Lane 2: 2 ng of the Procathepsin K. **Dot Blot** of HRP using this kit (**C)** and competitor's kit (**D**). HRP was spotted on PVDF membrane starting at 10 ng and serially diluted 1:1 subsequently. The membrane was exposed to G:BOX for 3 min. **Western Blot** of protein SAHH (Cat # 7527) was performed using this kit (**E**) and competitor's kit (**F**). The protein was separated on 8% SDS-PAGE and transferred to PVDF membrane. Sample Loading: 0.1, 0.2, 0.5, 1, 2, 5, and 10 ng (from left to right).

The blot was incubated with 4 ug/ml anti-rabbit SAHH antibody (Cat # 6684) overnight at 4°C, followed by incubation with HRP-conjugated goat anti-Rabbit IgG at 1:5,000 dilution for 1 hr at room temperature. The membrane was exposed to G:BOX for 3 min. (**E**) and 10 min. (**F**).

VIII. RELATED PRODUCTS:

Western Blot Substrate Kit (K820)
BCA Protein Quantitation Kit (K812, K813, K814)
Protease & Phosphatase inhibitor cocktails (K283, K284)
Protein Quantitation kit (K810)
Protein Carbonyl Content Assay Kit (K830)
Protease inhibitor cocktails (K271, K272, K277, K278, K279)

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