Cat. No. 293

WestShow®

ECL Western Blotting Substrate Instructions

Introduction

The ECL Western blotting is a powerful and commonly used tool to identify a specific protein in a complex mixture. As shown in Fig. **1**., protein samples are first resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) and then electrophoretically transferred to the nitrocellulose or polyvinylidene fluoride (PVDF) membrane. Following a blocking step, the membrane is probed with a primary polyclonal or monoclonal antibody. After a subsequent washing step, the membrane is incubated with a secondary antibody conjugated to an horseradish peroxidase (HRP) enzyme. The secondary antibody reactive toward the primary antibody. After a final wash step, the membrane is incubated with this ECL Western Blotting Substrate assay kit to generate a recordable signal.

We developed this assay kit seriously, and compared with the competitor. The efficiency and sensitivity are much better than the counterpart (Fig. **2** and **3**.). We recommend this product to your sincerely.

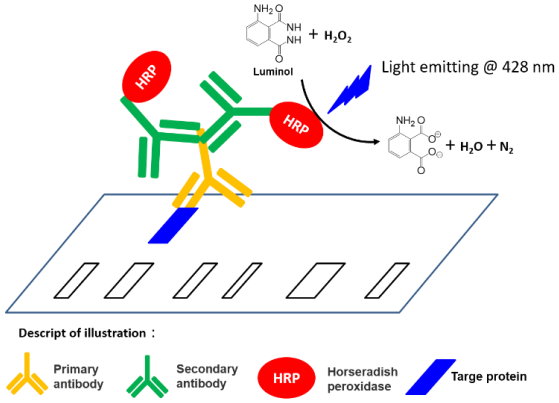

Fig. **1**. Principles of ECL Western blotting Protein Detection

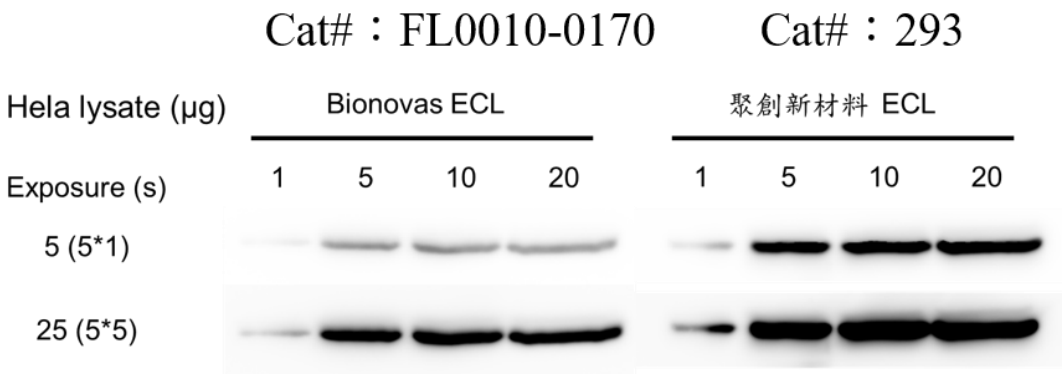

Fig. **2**. The comparison of our product and competitor.

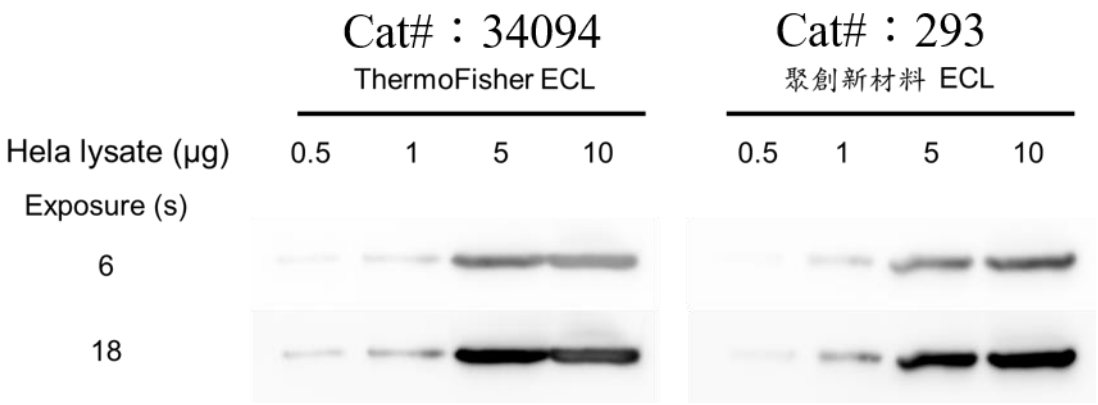

Fig. **3**. The comparison of our product and competitor.

Shipping and Storage Information

Product Name	shipping	Storage
WestShow (Cat. No.：293)	Shippimg under room temperature	6 months under 4 °C with protection from light.

Materials to be Supplied by the User

1. PVDF or NC blotted membrane.
2. TBS or PBS blocking buffer.
3. TBST wash buffer.
4. primary antibody.
5. HRP-conjugated secondary antibody.
6. tray for incubating and washing membrane.
7. rotary or rocking platform shaker.

Protocol

1. After protein transfer, remove membrane from the transfer apparatus, and block nonspecific sites with either Tris-buffered saline blocking buffer (TBS blocking buffer) or phosphate-buffered saline blocking buffer (PBS blocking buffer), Incubate for 1 hour at room temperature with shaking or at 4°C overnight without shaking.

Note: Milk may be substituted for BSA, depending on the primary；TBS blocking buffer：0.05–0.1 % Tween 20, 2–5% bovine serum albumin (BSA)；PBS blocking buffer：0.05–0.1% TweenR 20, 2–5% BSA.

2. Remove blocking solution, and add diluted primary antibody solution. Incubate for 1 hour at room temperature.
3. Wash 3 times, five minutes each wash, using TBST wash buffer：TBS and 0.05–0.1% Tween 20.
4. Incubate membrane with diluted secondary antibody solution (HRP conjugated) for 1 hour at room temperature with shaking.
5. Wash three times with TBST, 5 minutes each wash. Additional washes may help minimize background.
6. Prepare the substrate working solution：mix equal volume of Reagent A (black plastic bottle) and Regent B (white plastic bottle). Mix just enough working solution to cover the membrane. (e.g., 6–7ml per 10 X 5 cm membrane).

Note: use the prepared substrate working solution immediately after mixing. The solution is stable for up to 1 hour at room temperature.

7. Incubate the membrane for 1~5 minutes at room temperature without shaking. **The working solution need to cover the membrane.**
8. Remove the membrane from solution, blot excess liquid with an absorbent towel, and place in a plastic sheet protector.
9. Working in a dark room with a safe light, place covered membrane in a film cassette with protein side facing up. Place film on top of membrane, and

expose for 1 minute. Exposure time can be increased to achieve optimal results, with light emission being most intense immediately after substrate incubation and significantly decreasing within several minutes.

Troubleshooting

Problems	Causes	Suggestions
White bands with a black background	Too much HRP in the system	Dilute HRP-conjugate further
Membrane has brown or yellow bands		
Blot glows in the darkroom		
Weak or no signal or signal fades quickly	Too much HRP exhausted the substrate	Dilute HRP-conjugate further
	Used insufficient quantities of antigen or antibodies	Strip and re-probe blot using increased amount of antibodies
	Inefficient protein transfer	Optimize transfer conditions
	Low HRP activity	To test system activity, in a darkroom, prepare 1-2mL of the substrate working solution in a clear test tube. With the lights turned off, add 1µL of undiluted HRP-conjugate to the working solution. The solution should immediately emit a blue light that fades during the next several minutes.
High background	Too much HRP in the system	Dilute HRP-conjugate further
	Inadequate blocking or used inappropriate blocking reagent	Optimize blocking conditions
	Inadequate washing	Increase duration, number and volume of washes
	Overexposed film	Decrease exposure time
	Used too much antigen and/or antibody	Strip and re-probe blot using decreased amount of antibodies
Spots with the protein bands	Inefficient protein transfer	Optimize transfer procedure
	Unevenly hydrated membrane	Hydrate membrane according to manufacturer's instructions
	Bubble between X-ray film and membrane	Remove all bubbles before exposing blot to film
Speckled background	Aggregate formation in the HRP-conjugate	Filter HRP-conjugate through a 0.2µm filter before use
	Over-heating during electrophoresis or transfer	Control temperature during electrophoresis and transfer
Nonspecific bands	Too much HRP-conjugate	Strip and re-probe blot using a more dilute HRP-conjugate
	SDS caused nonspecific binding to protein	Do not use SDS during immunoassay procedure

***特別感謝國內多所研究機構協助本產品測試並提供相關測試數據。**

***感謝國立清華大學生命科學系選用本產品。**

***感謝國立成功大學醫學院生化所選用本產品。**

***感謝長庚醫院選用本產品。**

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