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ECL Western Blot Detecting Kit

(JZ-010)

ECL Western Blot Kit is a ready substrate to be widely used for chemiluminesecent detection on **Western blot**- immobilized protein conjugated with HRP directly.

Principle of ECL detection

In the presence of hydrogen peroxide (H_2O_2),), Horseradish peroxidase (HRP) catalyzes Following the oxidation reaction, as the intermediate reaction product, it is in an excited state, which decays to the ground state by emitting light. The strong enhancement of the light emission is produced by enhancers, such as Phenolic compounds. According to the principle, a new formulation- the ECL Western were designed to detect membrane immobilized specific antigen.

Highlights

1. The kit price is lower than the cost of any ECL reagent from other company.
2. High sensitivity; You could have the good results on the 5-40 ug /well protein loading, see the **Figs** below:

Fig-1: Protein Loaded (ug /well)

5 10 20 40

Exposure

10 seconds

20 seconds

30 seconds

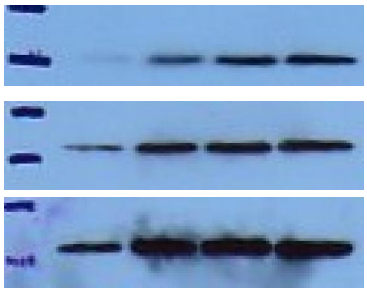
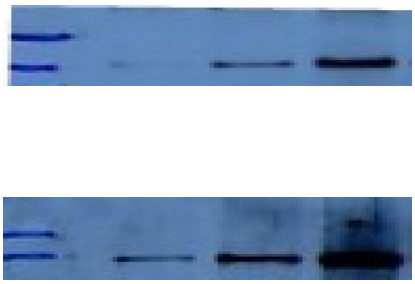


Fig.-2: Protein Loaded (ug /well)

10 20 40



The protein was from 293 cells, which were separated by electrophoresis. The proteins were transferred to nitrocellulose membranes and the membranes (Fig**-1**) were incubated with anti-Actin (42 **Kda),** Fig**.-2** were incubated with Anti- Sirt1 (**120 Kda**), no blocked, at RT for 20 Minutes, then, were incubated with HRP-conjugated IgG at RT For 20 minutes too (Half Hour Western Blot Kit, Cat#; JZ-009). Each membrane was detected with ECL Western Blot Detecting Kit **(JZ-010)**.

Content Table

Cat#	Name	Volume	Working for	Price/kit($)	Storage
JZ-010	ECL Reagent-1	250 ml	2500 cm^2 (0.2ml/ cm^2)	99.80	From Open Day: At 4˚C for 0.5-1 year; At RT for 3-6 month
	ECL Reagent-2	250 ml			

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Quick Protocol:

1. * Mix an equal volume mixture of ECL Reagent 1 and 2 to give enough solution to cover the membrane (0.15-0.2 ml/cm^2). Let the mixture equilibrate for 5-10 seconds. It is ready ECL mixture.
   *doing the process under the nature light. Or in dark Room under the red safely light.
2. Place the Western Membrane, washed well, in the ready ECL mixture, and make sure the protein side is up, and covered inside the ECL mixture, shaking at RT for 30-60 seconds.
3. Take out the membrane and to drain off excess ECL mixture with paper towel. Wrap the membrane in saran wrap. Gently remove air pockets.

It (1-3 above) would be better to be processing in dark room or in Room without light.

4. Place the membrane, protein side up, in the cassette; place a film on the membrane to be exposed for 10-30 seconds under the dark or with the red safety light. Then to be developing in the machine.
5. If the signal was too low, you could keep the exposure at RT for 30 minutes. Then to be developed again.
6. If the signal intensity was too high: 1). you could keep the filmfor 5-25 minute in dark, then to be developed again. 2). you also to dilute the 1/3-1/5 ECL mixture in wash buffer to get the desired results.

References:

1. Tsukagoshi K, et al.: Enhancing effect of phenylboronic acid compounds and their interactions with the diol groups of saccharides in a capillary electrophoresis-chemiluminescence detection system. Anal Sci., 2007 Feb; 23(2): 227-30.

2. Alpeeva IS, rt al.; Luminol-hydrogen peroxide chemiluminescence produced by sweet potato peroxidase. Luminescence, 2007 Mar-Apr; 22(2): 96-6.

3. Semenkova GN, et al.; Chemiluminescence in the peroxidase oxidation of luminol with hydrogen peroxide in various media. Lab Del; 1991(11): 13-5

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