ECLiNano™ ECL Chemiluminescence Kit

Catalog # ECL01-01

GeneBio Systems, Inc.™

Product	Cat. #	Size
ECLiNano™ ECL Chemiluminescence Kit	ECL03-01	100 mL (50 mL +50 mL)
	ECL03-02	200 mL (100 mL +100 mL)

Product Description

This product is a sensitive chemiluminescence kit. The basic principle is luminol-based chemiluminescence; it can react with horseradish peroxidase (HRP) coupled to the secondary antibody to emit light. Detection by X-ray film exposure or other imaging methods (such as fluorescence or chemiluminescence imager); with mid to high-concentration samples, signals can still be detected, saving samples; low-concentration antibodies (primary antibody, secondary antibody) can still detect signals after incubation, saving precious antibodies.

Shipping and Storage

Ship on ice or blue ice pak; store at 4℃

Kit Composition

Component Number	Component	ECL01-01 (total of 100 mL)	ECL01-02(total of 200 mL)
ECL03-A	ECLiNano A Solution	50 mL	100 mL
ECL03-B	ECLiNano B Solution	50 mL	100 mL

Procedure

1. Mix equal volumes of ECL03-A solution and ECL03-B solution (1:1) to make ECL working solution, prepare it at room temperature, and use it immediately.

2. Follow your procedure of Western Blot experiment, to the step in which the PVDF membrane (or NC membrane) is incubated with the secondary antibody. Washed the blot several times,

and the excess liquid is absorbed by the filter paper. Place the membrane between two pieces of clean plastic wrap (or PE gloves). Add ECL working solution to cover the surface of the membrane. This process should be done carefully to avoid air bubbles between the membranes and the wraps.

3. After allowing for sufficient time for the reaction to proceed, use filter paper or absorbent paper to absorb excess ECL working solution, and then carry out film exposure or detection by a fluorescence imager. Adjust detection settings on your imager to suite the need of your detection. Adjust exposure conditions according to luminous intensity being observed.

Precautions

1. Be sure to replace the pipette tip during the pipetting process of ECL solution A and solution B to avoid cross-contamination of liquid A and liquid B, which may cause the active ingredients to fail.

2. When contacting with the membrane, please wear gloves and use clean equipment such as clean tweezers to avoid contamination by exogenous proteins and metal ions.

3. Sodium azide can inhibit the activity of HRP, and thus should be avoided in all related reagents used in this procedure.

4. After the ECL working solution is prepared, please use it up within one day, and do not keep it until the next day, so as not to compromise the accuracy of the experimental results.

5. After each solution is used, please close the cap tightly and store it away from light to prevent failure; especially B solution, which contains oxidizing agents, is easy to be reduced and lose effectiveness.

6. For the selection of ECL chemiluminescence kit, please refer to the attached Table 1. The recommended dilution ratio test data of primary antibody and secondary antibody are all from self-developed antibodies.

7. For common problems and their solutions of ECL chemiluminescence detection, refer to Table 2.

8. ECL chemiluminescence reagent kit A/B solutions are harmful to human body, please be careful when operating, pay attention to effective protection, avoid direct contact with human body or inhalation of respiratory tract-wear lab coats and gloves

9. For your safety and health, please wear a lab coat and disposable gloves for operation.

Table 1 ECL Substrate reagent selection guide

Product	**ECLiNano™ ECL Chemiluminescence Kit**	**ECLiPico™ ECL Chemiluminescence Kit**	**ECLiFemto™ ECL Chemiluminescence Kit**
Cat. #	ECL03-01	ECL02-01	ECL01-01
Detection Limit	Nanogram level	Picogram	Femtogram
Primary antibody dilution (1 mg/ml stock)	1:1000-1:5000	1:4000-1:10000	1:10000-30000
Secondary antibody dilution (1 mg/ml stock)	1:1000-1:6000	1:3000-1:60000	1:6000-150000
Features	Wide range and applicability	Wide range; versatile; high sensitivity, for low to mid abundance protein, no need for large amount of antibody	Very high sensitivity; low abundance protein, suitable for working with expensive or limiting amount of antibody

Table 2 Common problems and recommendations in ECL detection

Problem	**Possible Cause**	**Recommendation**
High background (with/without specific bands)	Incorrect primary or secondary antibody or incorrect dilution	Make more antibody more diluted
	Incorrect blocking or insufficient blocking	Must use BSA or a protein-free blocker for detection of phosphorylated protein
	Insufficient incubation with primary antibody	Lengthen the incubation time (consider overnight at 4° C)
Weak signal	Concentration of primary or secondary antibody too low	Increase concentration of primary or secondary antibody
	Protein abundance is too low	1. Increase volume of sample in gel electrophoresis 2. Use the femto grade ECL if appropriate.
Light quickly quenched, bands empty in the middle (ghost bands)	Bands are too intense, ECL substrates are depleted quickly.	Reduce sample volume in gel and/or amount of primary or secondary antibody

Brown or yellow color bands on membrane	Bands or areas with too much HRP enzymatic activity, resulting too much free radicals, inactivating failure in oxidation	Reduce sample volume in gel and/or amount of primary or secondary antibody