



Pico ECL Chemiluminescent Substrate Specification

Product number: DIB050-100ml DIB050-500ml
Product specifications: 100ml 500ml

Storage: Transport at room temperature, store reagents at 4° C and protected from light after receipt

The dilution range of the primary antibody is from $\mu\text{g/ml}$ stock solution 1:1000-1:5000 or 0.2-0.1ng/ml

The dilution range of the secondary antibody is from $\mu\text{g/ml}$ stock solution 1:20000-1:100000 or 10-50ng/ml

Table 1: Antibody dilution range used with Pico ECL chemiluminescent substrate

Product Introduction

The Pico ECL substrate can provide a bright signal for western blotting experiments using horseradish peroxidase (HRP) conjugates. Thanks to the use of Fubaike's unique core patented technology, a luminescent substrate system designed based on the "ping-pong" mechanism. HRP can achieve low picogram and femtogram level western blot detection, and its sensitivity, luminescence time and background are significantly better than T company and M company. The ECL substrate is compatible with various membranes, blocking solutions and a wide range of antibody diluents. With excellent performance, versatility and high cost performance, it can meet the needs of users for western blotting applications.

Features of PicoECL substrate:

- PicoECL—enhanced chemiluminescence substrate for horseradish peroxidase (HRP)
- Low picogram sensitivity—detects low picogram protein bands on nitrocellulose membrane or PVDF membrane
- Long signal duration — Under optimized conditions, the blot strips incubated with the substrate can continuously output a detectable light signal for 6 to 8 hours.
- Stabilizing reagent — The working solution remains stable within 24 hours; the kit can be stored stably at room temperature for up to 1 year
- Economical price—optimized for diluted antibody concentration conditions:
 - 0.2 to 1.0 $\mu\text{g/mL}$ primary antibody (diluted 1:1,000 to 1:5,000 times with 1 $\mu\text{g/mL}$ stock solution)
 - 10 to 50 ng/mL Secondary antibody (diluted 1:20,000 to 1:100,000 times with 1 $\mu\text{g/mL}$ stock solution)

important hint:

Ø For best results, all components of the system must be optimized, including sample volume, primary and secondary antibody concentrations, and types of membranes and blocking reagents.

Ø The antibody concentration required for the detection of this product is lower than that of the precipitation colorimetric HRP substrate. To optimize antibody concentration, perform a systematic dot blot analysis.

Ø No blocking reagent is the best for all systems, so it is very necessary to find the most suitable blocking buffer for each western blot detection system. The blocking reagent may cross-react with the antibody, resulting in non-specific signals. The blocking buffer also affects the sensitivity of the system. When switching from one substrate to another, signal attenuation or background increase sometimes occurs. The reason may be that the blocking buffer is not suitable for the new detection system.

Ø When using avidin/biotin detection system, avoid using milk as a blocking reagent, because milk contains unquantified endogenous biotin, which will cause high background signals.

Ø Ensure the use volume of washing buffer, blocking buffer, antibody solution and substrate working solution to ensure that the blotting membrane is completely covered by the liquid during the entire experiment and to prevent the membrane from drying out. Increasing the amount of blocking buffer and washing buffer used can reduce non-specific signals. For best results, use a shaker during the incubation step.

Ø Add Tween20 (final concentration 0.05-0.1%) to blocking buffer and diluted antibody solution to reduce non-specific signals. Use high-quality products such as detergents. It is stored in ampoules, and the content of peroxides and other impurities is very low.

Ø Do not use sodium azide as a preservative in the buffer. Sodium azide is an inhibitor of HRP. Avoid direct contact between hands and the membrane. Wear gloves or use clean tweezers during the experiment. All equipment must be clean and free from foreign substances. Metal instruments (such as scissors) must not have visible rust. Rust may cause spot formation and high background.

Ø The substrate working solution can be stable for 8 hours at room temperature. Sunlight or any other strong light may damage the substrate. For best results, store the substrate working solution in an amber bottle and avoid long-term exposure to any strong light and short-term exposure to the laboratory's routine lighting. Will not damage the working fluid

Operation overview

Note: Optimize the concentration of antigen and antibody. The recommended antibody dilution must be used to ensure a positive result. For the recommended dilution range, please refer to other required materials.

- 1) Dilute the concentration of the primary antibody to 0.2- $\mu\text{g/ml}$
- 2) Dilute the concentration of the secondary antibody to 10-50ng/mL
- 3) Mix the two substrate components at a ratio of 1:1 to prepare the substrate working solution.

Note: Exposure to sunlight or any other strong light may damage the working fluid. For best results, store this working fluid in an amber bottle and avoid long-term exposure to any strong light. Exposure to routine lighting in the laboratory for a short time will not damage the working fluid.

- 4) Incubate the blotting membrane in West Pico ECL Substrate Working Solution for 5 minutes.

Other required materials

I The blotting membrane that has been transferred: Separate the proteins using a suitable electrophoresis method and transfer these proteins to the nitrocellulose membrane.

I Dilution buffer: use Tris or phosphate buffer.

I Washing buffer: add 5mL 10% Tween-20 to 1000mL dilution buffer (the final concentration of Tween-20 will be 0.05%).

I Blocking reagent: add 0.5mL of 10% Tween-20 to 100mL of blocking buffer, and select a blocking buffer with the same basic components as the dilution buffer.

I Primary antibody: Choose an antibody specific to the target protein. Use dilution buffer to prepare a 1 $\mu\text{g/ml}$ stock solution of the antibody. Use blocking reagent to dilute the antibody from the stock solution to the antibody working solution. The dilution is between 1:1000 and 1:5000 or the antibody working solution concentration is 0.2~1 $\mu\text{g/ml}$. The best dilution depends on the amount of the primary antibody and the antigen on the membrane.

I HRP-labeled secondary antibody: Choose a HRP-labeled secondary antibody that specifically binds to the secondary antibody, and use the dilution buffer to prepare a 1 $\mu\text{g/ml}$ stock solution of the antibody. Use blocking reagent to dilute the antibody from the stock solution to the antibody working solution. The dilution is between 1:20000 and 1:100,000 or the antibody working solution concentration is 10-50ng/ml. This concentration range also applies when using streptavidin-HRP. The optimal dilution of the secondary antibody depends on the HRP-labeled secondary antibody and the amount of antigen on the membrane.

I Film cassettes, developing and fixing reagents for processing radiographic films

I Rotary shaker for incubation.



Detailed steps of Western blotting

- 1) Take the imprinted membrane out of the protein transfer equipment, add a suitable blocking solution and incubate in the greenhouse for 20-60 minutes while shaking. To block non-specific protein binding sites on the membrane. Please note: it is very important to use the antibody dilution recommended in the previous section.
- 2) Take the membrane out of the blocking solution and incubate it with the working solution of the primary antibody in the greenhouse for 1 hour while shaking; or incubate overnight at 28° C without shaking.
- 3) Add enough washing buffer to the membrane to ensure that the buffer completely covers the membrane. Incubate with shaking for ≥ 5 minutes, change the washing buffer and repeat this step 4-6 times. Increasing the volume of the wash buffer, the number of washes and the washing time help to reduce the background signal. Note: Before incubation, a short rinse of the membrane in the washing buffer will improve the washing efficiency. Please note: It is very important to use the HRP-labeled secondary antibody dilution suggested above.
- 4) Incubate the HRP-labeled secondary antibody working solution with the membrane in the greenhouse for 1 hour while shaking.
- 5) Repeat step 3 to remove unbound HRP-labeled secondary antibody. Note: The membrane must be washed thoroughly after incubating with the HRP-labeled secondary antibody.
- 6) Mix A solution and B solution in equal proportions to prepare a working solution. Use 0.01~0.1ml working solution per cm² of membrane. The working fluid can be stable for 8 hours in the greenhouse. Note: Heavy rain, sunlight or any other strong light may damage the working fluid. In order to obtain the results of the mouth, store this working fluid in an amber bottle and avoid any strong light from long-term heavy rain. Common lighting in the laboratory will not harm the working fluid.
- 7) Incubate the imprinted membrane in the working solution for 5 minutes.
- 8) Take out the imprint film from the working fluid and place it in a plastic sheet or clean plastic paper (film). Use a piece of absorbent paper to absorb the excess liquid, and carefully press out bubbles from the imprint and the plastic paper.
- 9) Place the imprint film wrapped in plastic paper (film) in a film cassette with the protein side facing up, and turn off all lights except for the lights suitable for film exposure (such as red safety lights). Note: The film must be kept dry during the exposure. For best results, the following measures should be taken: * Ensure that the excess substrate is completely removed from the film and plastic paper. * During the entire film processing, use gloves. * Do not place the imprint film on the developed film, because the chemicals on the film will weaken the signal.
- 10) Place the X-ray film on top of the film. It is recommended that the first exposure is 60 seconds. The exposure time can be adjusted later to achieve the best results. The chemiluminescence reaction is strongest during the first 5-30 minutes after the substrate incubation. This reaction can last for several hours, but the intensity will decrease with time. If the substrate is exposed for a longer time after incubation, the exposure time may need to be extended to obtain a stronger signal. If you use phosphorescent storage imaging devices (such as Bio-Rad's molecular imager system) or CCD cameras, longer exposure times may be required. Warning: Any movement between film and film may cause artificial, non-specific signals on the film.
- 11) Use suitable developer and fixer to develop the film. If the signal is too strong, shorten the exposure time or peel off the imprinting film and reduce the

Common problems and solutions

problem	Possible problem	solution
There is a reverse image on the film (ie black background, white band)	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10 times
Brown or yellow bands on the membrane		
The imprint shines in the dark room		
Signal duration is less than 8 hours		
Weak or no signal	Too much HRP in the system depletes the substrate and causes the signal to decay rapidly	Dilute the HRP-labeled secondary antibody at least 10 times
	Insufficient amount of antigen or antibody	Increase the amount of antibodies or antigens
	Low protein transfer rate	Optimize transfer
	Low HRP or substrate activity	See note below
High background	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10 times
	Insufficient closure	Optimize sealing conditions
	Enclosed machine is not suitable	Try a different blocking reagent
	Insufficient washing	Add washing time, times or washing buffer volume
	Film overexposed	Reduce exposure time or use background remover
	The concentration of antigen or antibody is too high	Reduce the amount of antibodies or antigens
Spots in protein bars	Low protein transfer efficiency	Optimize the transfer process
	Uneven hydration of membrane	Properly hydrate the membrane according to the manufacturer's recommendations
	There are bubbles between the film and the film	Before film exposure, remove air bubbles
The film has spots on the background	There are aggregates in the HRP-labeled secondary antibody	Use 0.2um filter
Non-specific band	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10 times.
	Non-specific protein binding caused by SDS	SDS is not used during testing

*To detect the activity of the system, prepare 1-2ml of working solution of the substrate in a clean test tube in a dark room. Turn off the light and add 1ul undiluted HRP-labeled secondary antibody working solution. The solution should emit blue light immediately, and the blue light signal fades in the next few minutes.

Product parameter

Name	Pico ECL		
CAT#	DIB050-100mL;	CAS#	N/A
	DIB050-500mL		
Storage#	4° C dry and avoid light	Shelf Life#	12 months
Ex (nm) #	N/A	Em (nm) #	N/A
MW#	N/A	Solvent#	N/A