Data Invention Biotech

West Pico ECL Chemiluminescent Substrate Manual

Product number: DIB050-2-100ml DIB050-2-500ml

Product specifications: 100ml 500ml

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

Important note: West Pico chemiluminescence substrate is a highly sensitive substrate, its sensitivity is higher than other chemiluminescence products (including Thermo Scientifie, ECL), in order to obtain the best effect of the substrate, the antibody must be The concentration is lower when used together. If you are already using one of the above-listed substrates or another "entry level" chemiluminescent substrate, please dilute the antibody at least five times, for example, if you use ECL substrate, press 1 :100 dilution, then the antibody should be diluted 1:500 when using West Pico substrate. The recommended dilution range is listed below.
Primary antibody dilution range from 1mg/ml stock solution 1:1,000-1:5,000 or 0.2-0.1ug/ml
The dilution range of the secondary antibody is from 1mg/ml stock solution, 1:20,000-1:100,000 or 10-50ng/ml

Product Introduction

West Pico chemiluminescent substrate is a highly sensitive enhanced substrate for detecting horseradish peroxidase (HRP) on western blot membranes. The extremely strong signal output of the substrate enables the detection of picogram amounts of antigen. The sensitivity, intensity and duration of the signal make it possible to detect with photographic or other imaging methods. The blotting membrane can be exposed to the film repeatedly to obtain the best effect, or the immunoassay reagent on the membrane can be stripped and re-detected.

Important product information

★To obtain the best results, all components of the system must be optimized, including sample size, primary and secondary antibody concentrations, and types of membranes and blocking reagents. Because the substrate is extremely sensitive, West Pico substrate requires the use of much fewer samples, primary and secondary antibodies than most commercially available substrates, usually at least 10-20 times lower.

★The use of this product requires a lower antibody concentration than the use of precipitation colorimetric HRP substrate detection. To optimize antibody concentration, perform a systematic dot blot analysis. ★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. ★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. ★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 usage of blocking buffer and washing buffer can reduce non-specific signals. For best results, use a shaker during the incubation step. ★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 detergent. It is stored in ampoules, and the content of peroxides and other impurities is very low. ★Do not use sodium azide as a preservative for 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. ★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. Please refer to other required materials for the recommended dilution range.

1. Dilute the primary antibody from 1mg/ml stock solution to 0.2～1.0ug/ml or 1: 1.000～1:5.000 dilution
2. Dilute the secondary antibody from 1mg/ml stock solution to 10～50ng/ml or 1: 20.000～1:100.000 dilution
3. Mix the two substrate components in 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 blot membrane in West Pico Substrate Working Solution for 5 minutes.
5. Aspirate the excess reagent. Cover the blotting membrane with a clean plastic film.
6. Expose the blot film on X-ray film.

Other required materials

★ The blotting membrane that has been transferred: Use any suitable electrophoresis method to separate the proteins and transfer these proteins to the nitrocellulose membrane. Other types of membranes can also be used, but the operating steps may need to be optimized.
★Dilution buffer: use Tris or phosphate buffer.
★Washing buffer: Add 5mL 10% Tween-20 to 1.000mL dilution buffer (the final concentration of Tween-20 will be 0.05%). ★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.
★Primary antibody: Choose an antibody specific to the target protein. A 1 mg/ml stock solution of the antibody was prepared using dilution buffer. Use blocking reagent to dilute the antibody from the stock solution to the antibody working solution. The dilution is between 1:1.000 and 1:5.000 or the concentration of the antibody working solution is 0.2-1ug/ml. The best dilution depends on the amount of the primary antibody and the antigen on the membrane.
★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 1mg/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 1:20.000 and 1:100.000 or the concentration of antibody working solution is 10-50ug/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.
★Film cassettes, developing and fixing reagents for processing radiographic films
★Rotary shaker for incubation.

Detailed steps of Western blotting

1. Remove the imprinted membrane from 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 on other required materials.
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 washing buffer, the number of washes and the washing time can help reduce the background signal. Note: Before incubation, a short rinsing 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 recommended in the previous section on other required materials.
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. 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 imprinting membrane in the working solution for 5 minutes.
8. Remove 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 air bubbles from between the imprint and the plastic paper .
9. Place the imprint film wrapped in plastic paper (film) in the film cassette with the protein side facing up. 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. In order to obtain the best results, take the following measures: ensure that the excess substrate is completely removed from the film and plastic paper; use gloves during the entire film processing; do not remove the imprinted film Put it 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 the appropriate developer and fixer to develop the film.If the signal is too strong, shorten the exposure time or peel off the

Common problems and solutions

problem	Possible problem	solution
1) There is a reversal image on the film (ie black background, white band) 2) There is a brown or yellow band on the film 3) It is imprinted in the dark room to emit light 4) The signal duration is less than 8 hours	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10 times
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	4*See note below
	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 10 times
High background	Insufficient closure	Optimize sealing conditions
	Enclosed machine is not suitable	Try a different blocking reagent
	Insufficient washing	Increase washing time, number of 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 test the activity of the system, prepare 1-2ml of working solution of 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.