Data Invention Biotech

Femto ECL & Dura ECL chemiluminescent substrate manual

Product number: DIB051-2-100ml DIB051-2-500ml

Product specifications: 100ml 500ml

Kit components: ECL Solution A, 10mL; ECL Solution B, 10mL; Sufficient for 200cm2 of membrane

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

Important note:

Femto ECL & Dura ECL chemiluminescence substrate is an ultra-high sensitive substrate, and its sensitivity is higher than other chemiluminescence products (including ECL from Thermo and Millipore). In order to obtain the best effect of the substrate, The antibody must be at a lower concentration than the other substrates when used together.If you have used domestic ECL chemiluminescence solution, please dilute the antibody at least 10 times or more on the basis of the original dilution concentration. For example: if you use domestic ECL substrate to dilute the antibody at 1:100, then use Femto ECL & Dura ECL substrate antibody should be diluted 1:1000.Recommended dilution range.Primary antibody dilution range from 1mg/ml stock solution 1:1,000-1:5,000 or 0.2-0.1ug/ml Secondary antibody dilution range from 1mg/ml stock solution 1:20,000-1:100,000 or 10-50ng/ ml

Product Introduction

Femto ECL & Dura ECL 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 results, or the immunoassay reagent on the membrane can be stripped and re-detected.

Note (important):

★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, Femto ECL & Dura ECL substrate requires the use of much fewer samples, primary antibodies, and secondary antibodies than most commercially available substrates, usually diluted by 20-50 times.

★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.

★The blocking reagent may cross-react with the antibody, resulting in non-specific signals.The blocking buffer also affects the sensitivity of the system.It is very necessary to choose a suitable blocking buffer.

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

★The use volume of washing buffer, blocking buffer, antibody solution and substrate working solution must ensure that the blotting membrane is completely covered by the liquid during the entire experiment to prevent the membrane from drying out.Increasing the amount of blocking buffer and washing buffer used can reduce non-specific signals.

★Add Tween20 (final concentration 0.05-0.1%) into blocking buffer and diluted antibody solution to reduce non-specific signal.Do not use sodium azide, it is an inhibitor of HRP.

★For best results, use a shaker during the incubation step.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.Avoid exposure to ultraviolet and sunlight. Exposure to routine laboratory lighting for a short time will not damage the working fluid.

Overview of operation steps: 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/ultraviolet light will damage the working fluid. This working fluid should be stored in a brown bottle.Exposure to routine lighting in the laboratory for a short time will not damage the working fluid.4. Incubate the blot in Femto ECL & Dura ECL 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 best transfer these proteins to the nitrocellulose membrane.
★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.Note: It is very important to use the antibody dilution recommended above.
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: Exposure to sunlight/ultraviolet light will damage the working fluid. This working fluid should be stored in a brown bottle.Exposure to routine lighting in the laboratory for a short time will not damage 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 clean plastic sheet/film. Use absorbent paper to absorb the excess liquid, and carefully press out air bubbles between the imprint and the plastic paper.9. Put the imprint film wrapped in plastic paper (film) in the film cassette with the protein side facing up. Turn off all the lights except for the lights suitable for film exposure (such as red safety lights).Note: The film must be kept dry during the exposure; ensure that the excess substrate is completely removed from the film and plastic paper; during the entire film processing, use gloves; do not put the imprinted film on the developed film, because the film Chemicals 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 appropriate 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 antibody concentration to retest.

Detection sensitivity of different ECL luminescent fluids

product name	Detection concentration	Recommended primary antibody incubation concentration (ng/mL)	Recommended concentration of two-body incubation (ng/mL)
ECL plus	Pick class~10^{-12}g	Primary antibody concentration: 100～500	Concentration of secondary antibody: 10～50
Pico ECL	Pique to Feke10^{-12}g～0^{-15}g	Primary antibody concentration: 100～500	Concentration of secondary antibody: 10～50
Femto ECL	Less than femtogram<10^{-15}g	Primary antibody concentration: 20～100	Concentration of secondary antibody: 4～20
Dura ECL	Less than femtogram<10^{-15}g	Primary antibody concentration: 2～100	Concentration of secondary antibody: 2～10

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 20 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 20 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
High background	Too much HRP in the system	Dilute the HRP-labeled secondary antibody at least 20 times
	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 the membrane	Before film exposure, remove air bubbles
	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.