ECL Plus 化学发光底物 说明书

保存：室温运输，收到后在 4℃避光下储存试剂

一. 产品描述：Super ECL Plus超敏发光液用于检测直接或间接标记辣根过氧化物酶HRP的抗体及其关联的抗原。由于采用了独特的发光底物系统，Super ECL Plus超敏发光液是目前最灵敏的商业化荧光ECL检测试剂：

（1） 可使用更高的抗体稀释倍数(1:2000～1:10000)，极其节省抗体。

（2） 简单易用— 可替代其它公司的ECL发光底物，操作步骤无需进行特别优化

（3） 灵敏度更高— 可检测低皮克级的蛋白

（4） 信号持续时间更长— 光信号持续时间长达5小时

（5） 更多成像方法— 适用于X射线胶片、CCD或激光成像仪

（6） 价格更经济— 相比其他品牌的类似产品，不仅具有高品质和高性能，同时价格也更低

二. 用途：用于HRP标记抗体的Western Blot和HRP标记探针的核酸杂交。

三. 使用方法:

1. 执行常规SDS-PAGE、转膜和Western Blot步骤。注意用HRP标记IgG或用一抗-链亲和素-生物素-HRP夹法。

操作概述

注：优化抗原和抗体的浓度。必须使用建议的抗体稀释度，以保证阳性结果。

1) 将一抗浓度稀释到 0.05～1ug/ml
2) 将二抗浓度稀释到 0.005～0.04ug/mL
3) 将两种底物组份按 1:1 比例混合，制备底物工作液。
   注：暴漏于日光或任何其他强光可能损害工作液，为获得最佳结果，将此工作液保存在琥珀色瓶中，并避免长期暴漏于任何强光。短时间暴漏于实验室常规照明不会损害该工作液。
4) 将印迹膜在 ECL 底物工作液中孵育 5 分钟。
5) 吸出多余试剂。用清洁的塑料膜盖住该印迹膜。
6) 使印迹膜在 X 光胶片上曝光。

2. Western Blot最后一次洗膜的同时新鲜配制发光工作液：分别取等体积的溶液A和B，放入干净容器中混合。建议立即使用工作液，室温放置数小时后仍可使用但灵敏度略有降低。

3. 用镊子取出膜，搭在滤纸上沥干洗液但勿使膜完全干燥。将膜完全浸入发光工作液（0.125mL发光工作液/cm^2膜）中，与发光工作液充分接触。室温孵育3分钟，准备立即压片曝光。孵育时间过长不会增加灵敏度，有时还会导致曝光条带异常。发光过程的本质是酶促反应，使用过少的发光工作液不利反应进行，也会导致膜上条带曝光不均和明显降低灵敏度。为达节约目的可将膜剪小但勿降低发光液用量。

4. 用镊子夹起膜，搭在滤纸上沥干发光工作液。但勿洗去发光液。

5. 打开X光胶片暗盒，在暗盒内表面铺一张面积大于膜的保鲜膜。将Western Blot膜贴在保鲜膜上，将保鲜膜折起来完全包裹Western Blot膜，去除气泡和皱褶，可剪去边缘部多余的保鲜膜。用滤纸吸去多余的发光工作液。用胶带将覆盖Western Blot膜的保鲜膜固定在暗盒内，蛋白带面向上。

6. 暗房内压X光胶片，分别曝光不同的时间如数秒到数分钟。显影冲洗。

四. 储存：4 °C密封避光保存一年以上。短期可放置室温。

五. 安全性：无特殊毒性，按普通化学品处理。

六. 注意事项：

（1）步骤1～5可在日光灯下操作；但发光液曝露于强光下时间过久灵敏度可能略有降低，移到暗房操作可避免之。戴手套可以避免在膜上留下手印。

（2）长时间曝光或蛋白过量，将加深背景并使条带强弱变化失去线性关系。曝光不足则条带模糊。

（3）发光工作液孵育约3分钟后膜上的条带发光。强条带发光在暗房中肉眼可见，低丰度蛋白条带发光较弱甚至肉眼不可见但可使X光胶片曝光。不能简单以肉眼观察判断条带发光时间。肉眼不可见的荧光实际上可持续数小时并使X光胶片感光，因而弱带可曝光1-10小时。如果曝光后条带不佳，可用洗膜缓冲液洗膜，重新孵育二抗，然后重新用ECL发光和曝光。

（4）由于超敏发光液极其灵敏，强烈推荐大多数进口抗体起始浓度为一抗1:1000～1:4000，二抗1:2000～1:5000。抗体浓度过高将造成高背景或没有条带，导致失败。

（5）某些保鲜膜包裹印迹膜时可能会淬灭荧光，应选择高质量保鲜膜。

（6）使用肉眼可见的预染色蛋白Marker和荧光-放射自显影曝光标签可精确确定胶片上条带的位置和大小。

（7）NaN_3能抑制HRP活性，回收第二抗体应避免使用NaN_3，如必需使用勿超过0.01%。

ECL Plus substrate

Kit Contents: ECL plus Solution A, 50 mL; ECL plus Solution B, 50 mL; sufficient for 1000 cm2 of membrane

Storage: Upon receipt store solutions at 4°C. When stored at room temperature, substrate components are stable for six months. These products are shipped at ambient temperature.

Important Notice: Please use the same blotting conditions when switching from Thermo Scientific Pierce SuperSignal Western ECL plus Substrate or other similar ECL substrates. The ECL plus has been developed to be more sensitive compared to Western ECL plus Substrate or other similar ECL substrates. However, if you are currently using FBK Femto ECL substrate (Cat # F046), switching to FBK Pico Substrate may require increasing the antigen and antibody concentrations. Careful titration of antigen and antibody concentrations is recommended.

Introduction

The ECL plus Substrate is a highly sensitive nonradioactive, enhanced luminol-based chemiluminescent substrate for the detection of horseradish peroxidase (HRP) on immunoblots. The ECL plus Substrate enables the detection of sub-picogram amounts of antigen and allows for easy detection of HRP using photographic or other imaging methods. Blots can be repeatedly exposed to X-ray film to obtain optimal results or stripped of the immunodetection reagents and re-probed. The optimally formulated ECL plus Substrate makes it the ideal substitute for Pierce SuperSignal Western ECL plus substrate and other similar substrates without the need for additional optimization of assay conditions.

Important Product Information

1. For best results, it is ESSENTIAL to optimize all components of the system including sample amount, primary and secondary antibody concentration, and the choice of membrane and blocking reagents. Use the same blotting conditions when switching from Pierce SuperSignal Western ECL plus Substrate to ECL plus Substrate.
2. The antibody concentrations required are lower than those used with our competitors' similar products. To optimize the appropriate concentrations, perform a systematic dot blot analysis.
3. Because no blocking reagent is optimal for all systems, empirical testing is essential to determine the appropriate blocking buffer for each Western blot system. Determining the proper blocking buffer can help increase sensitivity and prevent nonspecific signal caused by cross-reactivity between the antibody and the blocking reagent. Furthermore, when switching from one substrate to another, a diminished signal or increased background sometimes results because the blocking buffer was not optimal for the new system.
4. Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin, which will result in high background.
5. Use a sufficient volume of wash buffer, blocking buffer, antibody solution and Substrate Working Solution to cover blot and ensure that it never becomes dry. Using large blocking and wash buffer volumes may reduce nonspecific signal.
6. For optimal results, use a shaking platform during incubation steps.
7. Add Tween®-20 (final concentration of 0.05%) to the blocking buffer and when preparing all antibody dilutions to reduce nonspecific signal.
8. Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
9. Do not handle membrane with bare hands. Always wear gloves or use clean forceps. All equipment must be clean and free of foreign material. Metallic devices (e.g., scissors) must have no visible signs of rust. Rust may cause speckling and/or high background.
10. The Substrate Working Solution is stable for 4 hours at room temperature. Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to laboratory lighting will not harm the Working Solution.

Procedure Summary

Note: The recommended range of antibody dilutions should be used as references to obtain positive results. The optimal antigen and antibody amounts to be used may require experimentation.

1. Dilute primary antibody to 100 - 500 ng/mL.
2. Dilute secondary antibody to 10 - 50 ng/mL.
3. Mix the two substrate components (A and B) at a 1:1 ratio to prepare the substrate Working Solution.

   Note: Exposure to the sun or any other intense light can harm the Working Solution. For best results keep the Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Short-term exposure to laboratory lighting will not harm the Working Solution.

4. Incubate blot 5 minutes in ECL plus Substrate Working Solution.

5. Drain excess reagent. Cover blot with clear plastic wrap.
6. Expose blot to X-ray film or Use imaging devices.

Additional Materials Required

1) Completed Western blot membrane: Use any suitable protocol to separate proteins by electrophoresis and transfer them to a membrane.
2) Dilution Buffer: Use either Tris-buffered Saline (TBS) or Phosphate-buffered Saline (PBS).
3) Wash Buffer: Add 5mL of 10% Tween-20 to 1000mL Dilution Buffer. (Final concentration of Tween-20 will be 0.05%.)
4) Blocking Reagent: Add 0.5 ml of 10% Tween-20 to 100mL of a blocking buffer. Choose a blocking buffer with the same base component as the Dilution Buffer.
5) Primary Antibody: Choose an antibody that is specific to the target protein(s). Use the Blocking Reagent to prepare a primary antibody working dilution ranging from 100 ng/mL to 500 ng/mL. For example, if the primary antibody is supplied at 1 mg/mL, dilute it in the range from 1:2,000 to 1:10,000. The necessary dilution to use depends on the specific primary antibody and the amount of antigen on the membrane and will require optimization for each experimental system.
6) Secondary Antibody: Use the Blocking Reagent to prepare a HRP-conjugate working dilution ranging from 10 ng/mL to 50 ng/mL. For example, if the antibody is supplied at 1mg/mL, dilute it in the range from 1:20,000 to 1:100,000. The necessary dilution varies depending on the primary antibody, HRP-conjugate and amount of antigen on the membrane
7) and will require optimization for each experimental system.
8) Film cassette, developing and fixing reagents: For processing autoradiographic film.(or
9) Imaging devices: e.g., Bio-Rad's Molecular Imager System or a similar gel documentation system.
10) Rotary platform shaker: For agitation of membrane during incubations.

Troubleshooting

Problem	Possible Cause	Solution
1)Reverse image on film (i.e., white bands with a black background) 2)Membrane has brown or yellow bands 3)Blot glows in the darkroom 4)Signal duration is less than 4 hours	Too much HRP in the system	Dilute HRP-conjugate
Weak or no signal	Too much HRP in the system depleted the substrate and caused the signal to fade quickly	Dilute HRP-conjugate
	Insufficient quantities of antigen or antibody	Increase amount of antibody or antigen
	Inefficient protein transfer	Optimize transfer
	Reduction of HRP or substrate activity	**See note below
High background	Too much HRP in the system	Dilute HRP-conjugate
	Inadequate blocking	Optimize blocking conditions
	Inappropriate blocking reagent	Try a different blocking reagent
	Inadequate washing	Increase length, number or volume of washes
	Film has been overexposed	Decrease exposure time
	Concentration of antigen or antibody is too high	Decrease amount of antigen or antibody
	Poor antibody specificity	Try different antibodies.
Spots within the protein bands	Inefficient protein transfer	Optimize transfer procedure
	Unevenly hydrated membrane	Perform manufacturer's recommendations for hydrating membrane properly
	Bubble between the film and the membrane	Remove all bubbles before exposing blot to film
Speckled background on film	Aggregate formation in the HRP- conjugate	Filter conjugate through a 0.2µm filter
Nonspecific bands	Too much HRP in the system	Dilute HRP-conjugate
	SDS caused nonspecific binding to protein bands	Do not use SDS during immunoassay procedure
	Poor antibody specificity	Try different antibodies.

**To test the activity of the system in the darkroom, prepare 1-2mL of the ECL plus Substrate Working Solution in a clear test tube. With the lights turned off, add 1µL undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes.