Lightgen™ CL Chemiluminescent Substrate

User's Manual

This instruction is suitable for following products.

Catalog No.	Name	Description	Volume
CHCL-250	Lightgen™ CL250	High Sensitive Chemiluminescent Substrate	250 ml
CHCL-125	Lightgen™ CL125	High Sensitive Chemiluminescent Substrate	125 ml
CHCL-050	Lightgen™ CL050	High Sensitive Chemiluminescent Substrate	50 ml

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A. Introduction

Lightgen™ CL is a chemiluminescent substrate with high sensitivity, using for detecting Horseradish Peroxidase in Western blot detection and Electrophoretic Mobility Shift Assay (EMSA). When incubating with HRP, it emits a blue light, which can be detected by X-Ray films or a CCD imager. Detecting sensitivity can reach to nanogram level of HRP on nitrocellulose (NC), PVDF or Nylon membranes. It may be necessary to optimize the dilution of antibodies, hybridization probes or HRP-conjugates to obtain best results. Please refer to the procedure section for details.

Lightgen™ CL is easy to use by combine equal amounts of Solution A and B.

B. Kit components

1. Lightgen™ CL 250 ml Substrate Kit – CHCL-250

   Lightgen™ CL Solution A, 125 ml
   Lightgen™ CL Solution B, 125 ml
   Sufficient for 150 pieces of (7 x 8 cm) membrane or ~8300 cm^2 membrane.

2. 125 ml Substrate Kit – CHCL-100

   Lightgen™ CL Solution A, 62.5 ml
   Lightgen™ CL Solution B, 62.5 ml
   Sufficient for 60 pieces of (7 x 8 cm) membrane or ~4000 cm^2 membrane.

3. 50 ml Substrate Kit – CHCL-050

   Lightgen™ CL Solution A, 25 ml
   Lightgen™ CL Solution B, 25 ml
   Sufficient for 30 pieces of (7 x 8 cm) membrane or ~1600 cm^2 membrane.

C. Additional materials required (not included):

Lightgen™ CL is designed for detecting the activities of HRP on nitrocellulose, PVDF or Nylon membranes. Before proceeding, please have the following materials ready.

1. Any one of the following completely reacted membranes:

   - In Western Blots, membranes should have been incubated with primary and HRP-conjugated secondary antibodies. They should be ready for chemiluminescent detecting.
   - In Northern and Southern Hybridization, membrane should have been hybridized with HRP-conjugated probes and ready for chemiluminescent detecting.
   - In EMSA, membrane should been incubated with detecting reagents and ready for chemiluminescent detecting.

2. X-rays films, film developer, and related chemicals and reagents, or a CCD image-detector, specifically for pickup Chemiluminescent signals, such as Viagene's Cool Imager™.

D. Procedures

1. Detection by exposing to X-rays films**:** When using X-rays films, make sure a dark room and a X-ray film developer are available, also have enough X-ray films, chemicals and reagents.
   1) Turn on and pre-warm the X-ray film developer.
   2) Make application mixture for membranes.

Solution A	1 ml
Solution B	1 ml
Total	2 ml

      *2 ml of substrate mixture is enough to cover a mini-gel membrane. A large membrane needs more substrate mixture. Averagely, 0.03ml of substrate mixture is enough to cover 1 cm^2 of NC, PVDF or Nylon membranes.
   3) Cover the entire membrane evenly with the substrate mixture and let it reacts at room temperature for 1-2 minutes.
   4) After the reaction, discard the substrate on the membrane and then cover it with a clear plastic film.
   5) In the dark room, put a sheet of X-rays film on the wrapped membrane. Since it is difficult to pre-determine the exposure time for each experiment, it may be necessary to try different exposure time to obtain best results.
2. Detection with a CCD Imager: For capturing chemiluminescent images, a CCD imager must have very high sensitivity and capacity to handle thousands of images in a short-time (1-15 minutes). Viagene's Cool Imager™ is a good example of choice for capturing images of Western Blots, Northern/Southern hybridizations and EMSA. Detecting and analyzing images with the CCD imager is much easier than X-Ray films.

   Following operation manual is for Viagene's Cool Imager™.
   1) Turn on Cool Imager™ and pre-cool the system for 1-2 minutes.
   2) Mix enough amount of substrate mixture [refer to diction D.1.2]. For example, each 9 X 7 cm membrane needs at least 2.0 ml total volume of the mixture.
   3) Place the membrane on a leveled and sturdy hydrophobic surface such as on a piece of a parafilm with a piece of glass under. Cover the entire membrane evenly with the substrate mixture.
   4) Make sure the membrane is covered with the substrate mixture evenly and then put the membrane with substrate solution into the imager.

5) Follow the detail instructions in Cool Imager™ user's manual. In general, 4-10 minutes of capturing should be sufficient to obtain a chemiluminescent image. Adjust the capturing time to obtain optimal images.

6) Use the Cool Imager™ Software to analyze and quantify the image.

7) Refer to the user's manual of Cool Imager™ for all the details and other functions.

E. Precautions

1. In order to obtain best results, it is important to use high-quality experiment materials, equipments, such as samples quality, probes and antibodies concentration, membrane quality, etc.
2. Before the experiment, it is recommended to check the concentration of probes and antibodies by performing the dot-blot analysis.
3. All the containers used in chemiluminescent experiments should be cleaned thoroughly. Always wear gloves or use cleaned forceps to handle membranes to avoid high background. Don't let membranes dry out during experiments.
4. Choose an appropriate blocking buffer. There is not a certain generic buffer for all Western blot, Northern and Southern hybridization, and EMSA. Choosing the appropriate blocking buffer may increase chemiluminescent sensitivity and reduces nonspecific background.
5. Avoid using milk in blocking buffer when using the avidin/biotin systems because milk contains variable amounts of endogenous biotin.
6. Adding Tween®-20 or other high quality detergent to the blocking and washing buffers may reduce nonspecific background.
7. Washing and blocking buffers cannot contain sodium azide as it will inhibit HRP activity.
8. Because the chemiluminescence decays over time, the first 5-30 minute reaction of HRP and chemiluminescent substrate produces the strongest chemiluminescence. In order to obtain best results, capturing chemiluminescent images with different exposure time is necessary.
9. During detection of chemiluminescent imaging, any movement of CCD camera or X-rays films on the membrane should be avoided to obtain best results.
10. Solution A and B should be stored in their own bottles at 4°C for long-term storage. At room temperature, the substrate mixture is stable for ~72 hours and exposure it to direct light may reduce its stability. However, short-time exposure to lab lighting has no effects on substrate solutions.

F. Troubleshooting:

Problems	Possible Causes	Recommendation
White spots in the center of dark chemiluminescent bands.	Too much HRP on sample area digests the substrate quickly and diminish chemiluminescence.	Dilute Probes, antibodies or HRP-conjugates more.
Brown or yellow spots on the membrane		
Very weak or no signal	Insufficient interaction of antigen & antibody	Increase amount of antigen or antibody
	Insufficient hybridization	Increase amount of RNA/DNA and probes
	Inefficient protein transfer	Optimize protein transfer
	Low HRP or substrate activity	Increase HRP-conjugates
High background	Too much HRP	Dilute HRP-conjugate more
	Insufficient blocking	Optimize blocking conditions
	Inappropriate blocking buffer	Try another blocking buffer
	Inadequate washing	Increase washing time and/or volume of washing solution
	Film or CCD camera have been overexposed	Decrease exposure time
	Concentration of antigen or antibody is too high	Reduce amount of antigen or antibody used in experiments.
White spots on the membrane randomly.	Inefficient protein transfer	Optimize protein transfer procedure
	Unevenly hydrated membrane	Follow manufacturer's recommendations.
	Bubbles between film and the membrane	Remove all bubbles before film exposure.

Speckles on the film background	Aggregate formation in the HRP-conjugate	Filter conjugates through a 0.2 μm filter
Nonspecific bands	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
	SDS caused nonspecific binding to protein bands	Do not use SDS during immunoassay procedure

G. Related Viagene Products

IMGR002 Cool Imager™ chemiluminescent imager workstation

TFDET001 Non-radioactive NF-KB EMSA Kit (40 reactions)

TFDET002 Non-radioactive STAT1 EMSA Kit (40 reactions)

TFDET003 Non-radioactive STAT3 EMSA Kit (40 reactions)

TFDET004 Non-radioactive STAT5 EMSA Kit (40 reactions)

TFDET005 Non-radioactive AP-1 EMSA Kit (40 reactions)

TFDET006 Non-radioactive HIF-1 EMSA Kit (40 reactions)

H. Warranty

Thank you for choosing Viagene products. Viagene Biotech Inc. guarantees that EMSA kit will perform as they are intended for and indicated in each manual. Products are warranted for 1 year from the date of purchase unless otherwise specified. During the warranty period, if problems arise, Viagene will be responsible for products exchange or return. All Viagene products are for research purposes only. No resale, distribution or sale modified products of Viagene Biotech, unless written permission is granted. Viagene Biotech Inc. strives for complete customer satisfaction, if you are not satisfied with Viagene's products or would like to give us comments, please contact Viagene Biotech customer service.

I. References

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