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ProductInformation

Chemiluminescent Peroxidase Substrate

Product Codes **CPS-1, CPS-1-30, CPS-1-60, CPS-1-120, and CPS-1-300**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description
Sigma's Chemiluminescent Peroxidase Substrate can be used for the highly sensitive detection of peroxidase labeled material in a variety of Western blotting applications. This substrate is an enhanced luminol product with a stabilized peroxide buffer solution that provides picogram sensitivity with minimal background interference.

Components
The Chemiluminescent Peroxidase Substrate is available in 4 package sizes each containing the Chemiluminescent Reagent (Product Code C 9107) and the Chemiluminescent Reaction Buffer (Product Code C 9232).

Package Size	C 9107	C 9232
30 ml	10 ml	20 ml
60 ml	20 ml	40 ml
120 ml	40 ml	80 ml
300 ml	100 ml	200 ml

Precautions and Disclaimer
This product is for laboratory research use only. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions
Prepare the Working Solution by mixing 1 part of the Chemiluminescent Reagent (Product Code C 9107) with 2 parts of the Chemiluminescent Reaction Buffer (Product Code C 9232). Mix well and protect from light. It is recommended to use 0.043 to 0.125 ml per cm^2 of membrane. For extended signal duration, a 1:1 ratio of Chemiluminescent Reagent to Chemiluminescent Reaction Buffer may be used.

Storage/Stability
It is recommended to store the components at 2-8 °C. The components are stable for a minimum of 18 months when stored in the original container and protected from light. The Working Solution is stable for several hours at room temperature when protected from light.

Procedure
Sigma's Chemiluminescent Peroxidase Substrate is very sensitive and great care must be taken to optimize the individual assay components (antibodies, conjugates, etc). In a Western blot, an optimized system is needed to minimize background reactivity associated with nonspecific immunochemical interactions. The following is a general guideline for the use of this product. The protocol starts with a transferred membrane.

Notes:
- For optimal results, individual assay components must be optimized for minimal background and maximal signal.
- This product is designed for use only in Western blotting.
- All steps below should be performed with slight agitation on a rocker or an orbital shaker such that the membrane is freely floating.
- All incubations should be performed at room temperature.
- Gloves must be worn when working with the membrane to avoid contamination.
- Azide inhibits horseradish peroxidase (HRP) and should not be used as a buffer preservative for assay components.

1. Remove membrane from Western blotting apparatus and wash membrane for 1 minute in either Tris-buffered Saline with TWEEN® 20 (TBST, Product Code T 9039) or phosphate buffered saline with TWEEN 20 (PBST, Product Code P 3563). Note that either a TBS or PBS system can be used for Western blotting.

2. Block membrane in appropriate blocking agent for 30 minutes. Western Blocker Solution (Product Code W 0138) is recommended for high sensitivity detection.

3. Add primary antibody to the blocking agent. The final concentration of primary antibody in this solution can range from 0.2-20 µg/ml.

4. Incubate membrane with the primary antibody solution for at least 30 minutes.

5. Wash with TBST or PBST for 1 minute.

6. Remove TBST or PBST and add at least 10 ml of appropriate blocking agent to the membrane. Add secondary antibody; a 1:50,000 to 1:500,000 dilution in blocking agent may be used.

7. Incubate the membrane with the secondary antibody solution for 30 minutes.

8. Remove blocking solution and wash membrane 5 times for 5 minutes each with TBST or PBST.

9. Remove the membrane from the wash buffer and drain any excess liquid from the membrane. Keep the membrane damp; do not let the membrane dry out.

10. Place the membrane on a flat sheet of plastic wrap (or on any clean plastic surface).

11. Develop the blots with the Working Solution for 5 minutes.

12. Drain excess substrate and place in holder or plastic wrap.

13. Expose BioMax light film to the blot. Exposure times range from 30 seconds to 10 minutes. It is best to do a quick exposure of 10 to 30 seconds to determine what exposure time is needed. If the signal is too intense even at the short exposure times, let the signal decay from 1 to 8 hours and then re-expose the film.

Related Products

Product Name	Package Size	Product Code
TBS	10 packets	T 6664
PBS	10 packets	P 3813
Western Blocker Solution	400 ml	W 0138
TBS + 3% milk	10 packets	T 8793
PBS + 3% milk	10 packets	P 2194
PBS + 5% milk	10 packets	P 4739
TBS + TWEEN 20	10 packets	T 9039
PBS + TWEEN 20	10 packets	P 3563
Anti-Mouse HRP Antibody	2 ml	A 9044

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Troubleshooting Guide

Problem Type	Cause	Solution
Too much background signal observed.	Not enough wash steps were performed at the end of the blotting.	Double the number of washing steps.
	Too much primary antibody used.	Lower the amount of primary antibody used and wash with TBST for 5 minutes instead of 1 minute after the primary antibody incubation.
	Too much secondary antibody used.	Lower the amount of secondary antibody used.
Image is reversed on film (dark background and light bands).	Too much secondary antibody used.	Lower the amount of secondary antibody used.
Bands on membrane have brown or yellow tone.	Too much secondary antibody used.	Lower the amount of secondary antibody used.
Nonspecific bands show up on membrane.	Too much primary antibody used.	Lower the amount of primary antibody used and wash with TBST for 5 minutes instead of 1 minute after the primary antibody incubation.
	Too much secondary antibody used.	Lower the amount of secondary antibody used.
Membrane is stippled.	Secondary antibody has some aggregate formation.	Filter secondary antibody.
No signal is seen with chemiluminescent reaction on membrane.	Protein levels are too low for detection.	Increase exposure time of film and increase level of protein loads.
	Not enough primary antibody used.	Use more primary antibody.
	Not enough secondary antibody used.	Use more secondary antibody.