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Product Information

Chemiluminescent Peroxidase Substrate-1

Catalog Numbers **CPS1A60, CPS1A120, and CPS1A300**
Storage Temperature 2–8 °C

Product Description

Chemiluminescent Peroxidase Substrate-1 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 femtogram sensitivity with minimal background interference.

Components

Chemiluminescent Peroxidase Substrate-1 is available in 3 package sizes each containing the Chemiluminescent Reagent (Catalog Number C9107) and the Chemiluminescent Reaction Buffer (Catalog Number C9232).

Package Size	C9107	C9232
60 mL	30 mL	30 mL
120 mL	60 mL	60 mL
300 mL	150 mL	150 mL

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Prepare the Working Solution by mixing equal volumes of Chemiluminescent Reagent (Catalog Number C9107) and Chemiluminescent Reaction Buffer (Catalog Number C9232) in a clean container and equilibrate at room temperature for 30 minutes before use. Mix well and protect from light.

Storage/Stability

The components are stable for a minimum of 18 months when stored in the dark at 2–8 °C, keep container tightly closed. The Working Solution is stable for several days at 4 °C when protected from light.

Procedure

The Chemiluminescent Peroxidase Substrate is extremely 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, Catalog Number T9039) or phosphate buffered saline with TWEEN 20 (PBST, Catalog Number P3563). 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 (Catalog Number W0138) 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). Optimal visualization may be obtained up to 20 minutes after substrate contact.

11. Develop the blots with the Working Solution for 5 minutes to 30 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 10–30 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 1–8 hours and then re-expose the film.

Related Products

Product Name	Package Size	Catalog Number
TBS	10 packets	T6664
PBS	10 packets	P3813
Western Blocker Solution	400 mL	W0138
TBS + 3% milk	10 packets	T8793
PBS + 3% milk	10 packets	P2194
PBS + 5% milk	10 packets	P4739
TBS + TWEEN 20	10 packets	T9039
PBS + TWEEN 20	10 packets	P3563
Anti-Mouse HRP Antibody	2 mL	A9044

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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.