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ProductInformation

Chemiluminescent Peroxidase Substrate-3

Product Codes **CPS-3-50, CPS-3-100, and CPS-3-500**
Storage Temperature 2-8 °C

TECHNICAL BULLETIN

Product Description
Chemiluminescent Peroxidase Substrate-3 (CPS-3) can be used for sensitive detection of peroxidase labeled materials in a variety of Western blotting applications. This substrate is an enhanced luminol-based product with a stabilized peroxide buffer solution, providing nanogram sensitivity with minimal background interference.

Components
The Chemiluminescent Peroxidase Substrate is available in 3 package sizes each containing the Chemiluminescent Reagent (Product Code C 7364) and the Chemiluminescent Reaction Buffer (Product Code C 7239).

Package Size	C 7364	C 7239
50 ml	25 ml	25 ml
100 ml	50 ml	50 ml
500 ml	250 ml	250 ml

Precautions and Disclaimer
This product is for R&D use only, not for drug, household, or other uses. 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 7364) with 1 part of the Chemiluminescent Reaction Buffer (Product Code C 7239). Mix well and equilibrate at room temperature for 30 minutes before use. It is recommended to use 0.043 to 0.125 ml per cm^2 of blotting membrane.

Storage/Stability
The components should be stored at 2-8 °C and are stable unmixed for a minimum of 18 months when stored in the original container and protected from light. After mixing, the Working Solution is stable for at least 45 days if stored in a tightly capped, light protected container at 2-8 °C.

Procedure
Chemiluminescent Peroxidase Substrate-3 is sensitive and care must be taken to optimize the concentration of 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 post-transfer Western blot membrane.

Notes:
- For optimal results, the concentration of individual assay components such as the primary and secondary antibody dilution must be optimized for minimal background and maximum signal.
- This product is designed for use only in Western blotting.
- Where appropriate, steps 1 through 8 below should be performed with gentle 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 any assay components.

1. Remove the membrane from the Western blotting apparatus and wash 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 the membrane with an appropriate agent, for at least 30 minutes with gentle agitation. For most routine applications, either TBS + 3% milk (Product Code T 8793), PBS + 3% milk (Product Code P 2194), or PBS + 1 % BSA (Product Code P 3688) are recommended.

3. Pour off the blocking solution. Dilute the primary antibody in fresh blocking solution and immediately add to the blot. The final concentration of primary antibody in this solution usually ranges from 0.2-20 µg/ml.

4. Incubate the membrane with the primary antibody solution for at least 30 minutes but no longer than 2 hours, employing gentle agitation at room temperature.

5. Pour off the primary antibody solution. Wash the blot 3-5 times for 5 minutes each with blocking solution, TBST, or PBST - to remove any unbound primary antibody.

6. Pour off the last wash. Dilute the secondary antibody-HRP conjugate 1:1,000 to 1:500,000 in fresh blocking solution and pour onto the blot.

7. Incubate the membrane with the secondary antibody-HRP conjugate solution for at least 30 minutes but no longer than 2 hours, employing gentle agitation at room temperature.

8. Remove the blocking solution containing the secondary antibody and wash the membrane 3-5 times for 5 minutes each with TBST or PBST.

9. The membrane should then be removed from the wash buffer and any excess liquid drained. Keep the membrane damp; do not allow the membrane to dry out.

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

11. Add Chemiluminescent Peroxidase Substrate-3 Working Solution and incubate for 5 minutes at room temperature without agitation. Agitation or excessive movement of the substrate may result in smearing of the substrate signal across the blot.

12. Quickly drain off any excess substrate and place the membrane in a holder, or wrap in plastic film.

13. Expose BioMax light film to the blot for times ranging from 5 seconds to 10 minutes. It is best to perform a quick exposure of 10 to 30 seconds to determine the exposure time needed. If the signal is too intense even at the short exposure times, allow the signal to decay for 15 minutes up to several 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
TBS + 3% milk	10 packets	T 8793
PBS + 3% milk	10 packets	P 2194
PBS + 5% milk	10 packets	P 4739
PBS + 1% BSA	10 packets	P 3688
TBS + TWEEN 20	10 packets	T 9039
PBS + TWEEN 20	10 packets	P 3563
Anti-Mouse IgG – Peroxidase	2 ml	A 9044
Anti-Rabbit IgG – Peroxidase	1 ml	A 0545

Troubleshooting Guide

Problem Type	Cause	Solution
Too much background signal observed.	An insufficient number of wash steps were performed after the primary and/or secondary antibody incubation.	Double the number of wash steps.
	Too much primary antibody used.	Lower the concentration of primary antibody used. Wash more frequently and/or for longer times after the primary antibody incubation.
	Too much secondary antibody used.	Lower the concentration of secondary antibody used. Wash more frequently and/or for longer times after the secondary antibody incubation.
Image is reversed on film (dark background and light bands).	Too much secondary antibody used.	Lower the concentration of secondary antibody used. Wash more frequently and/or for longer times after the secondary antibody incubation.
Bands on membrane have brown or yellow tone.	Too much secondary antibody used.	Lower the concentration of secondary antibody used. Wash more frequently and/or for longer times after the secondary antibody incubation.
Non-specific bands show up on membrane.	Too much primary antibody used.	Lower the concentration of primary antibody used. Wash more frequently and/or for longer times after the primary antibody incubation.
	Too much secondary antibody used.	Lower the concentration of secondary antibody used. Wash more frequently and/or for longer times after the secondary antibody incubation.
Membrane is stippled.	Secondary antibody has some precipitate formation.	Filter or centrifuge secondary antibody to remove precipitate.
No signal is seen with chemiluminescent reaction on membrane.	Unknown	Run a positive control of 1-10 ng/lane on the blot to ensure that the detection system is working properly.
	Protein levels are too low for detection.	Increase exposure time of film and increase level of protein loads.
	Not enough primary antibody used.	Use a higher concentration of primary antibody.
	Not enough secondary antibody used.	Use a higher concentration of secondary antibody.

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