PRODUCT INFORMATION & MANUAL

PicoTect™ Western Blot Chemiluminescent Substrate

NBP2-29912

Sufficient for 10 mini-blots (800 cm2 of membrane)

For research use only. Not fodiagnostic or therapeutic procedures.

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I. BACKGROUND

PicoTect™ Western Blot Chemiluminescent Substrate is a highly sensitive enhanced substrate for detecting horseradish peroxidase (HRP) on immunoblots. This substrate's extremely intense signal output enables detection of picogram amounts of antigen. The sensitivity, intensity and duration of the signal allows for easy detection of HRP using photographic or other imaging methods. Blots can also be repeatedly exposed to film to obtain optimal results or stripped of the immunodetection reagents and reprobed.

Catalog No	Component	Contents
NBP2-29912	PicoTect™ Substrate A	50 ml
NBP2-29912	PicoTect™ Substrate B	50 ml

II. KIT COMPONENTS AND STORAGE

The components included in PicoTect™ Western Blot Chemiluminescent Substrate may be stored at 4°C or RT for one year. Mix PicoTect™ Substrate A and PicoTect™ Substrate B at a 1:1 ratio to prepare the PicoTect™ Substrate Working Solution. The Working Solution is stable for 8 h at RT.

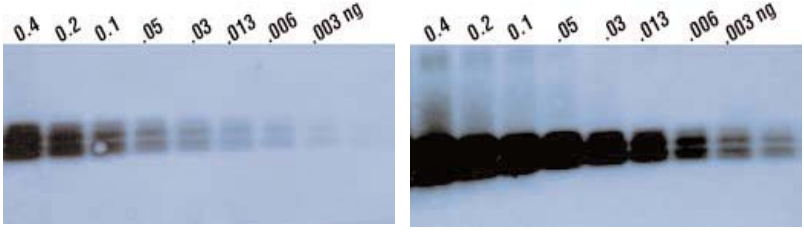
ADDITIONAL ITEMS REQUIRED (NOT INCLUDED IN THE KIT)

- **Immobilon P membranes (Millipore Corporation, MA)**. Nitrocellulose membrane or other completed western blot membrane types can be used; however, optimization may be required.
- **Wash Buffer**: TBST (25 mM Tris-Cl, pH 8; 125 mM NaCl; 0.1% Tween 20).
- **Dilution Buffer**: 1% Carnation nonfat dry milk in TBST.
- **Blocking Reagent**: 5% Carnation nonfat dry milk in TBST.
- **Primary Antibody**: Choose an antibody that is specific to the target protein(s) and prepare according to the manufactures specification.
- **HRP-conjugated Secondary Antibody (azide-free)**: Choose an HRP-conjugate that specifically binds to the primary antibody. The optimal dilution to use varies depending on the HRP conjugate and the amount of antigen on the membrane.
- **Film cassette, developing and fixing reagents**: For processing autoradiographic film.
- **Rotary platform shaker**: For agitation of membrane during incubations.

II. ADVANTAGES

PicoTect™ Western Blot Chemiluminescent Substrate is more sensitive than most chemiluminescent products including ECL™, LumiGLO®, Renaissance® and Western Lightning™ Substrates. For optimal performance in this system, antibodies must be more dilute than antibodies used with the other substrates previously mentioned. If you have been using one of these substrates dilute both primary and secondary antibodies at least 5-fold more. For example: If you have been using the primary antibody at 1:100 dilution with ECL™ Substrate, then use a ~1:500 dilution with this substrate. All antibodies purchased from Novus have been optimized using this system and do not need to be further diluted.

- **High intensity** signal– twice as intense as other luminol-based systems
- **Picogram sensitivity** – highly sensitive for the rapid development of a wide range of protein levels
- **Excellent stability** – 8-hour working solution stability



Comparison of sensitivity using ECL™ (A) and PicoTect™ Western Blot Chemiluminescent Substrate (B) detection systems. Two fold serial dilutions of protein were separated by SDS-PAGE and transferred to membrane. Film was exposed for 5 min.

PicoTect™ Western Blot Chemiluminescent Substrate has been tested on, and is compatible with, the following instruments:

Supplier	Instrument
Alpha Innotech	FluorChem and ChemiImager
Bio-Rad	ChemiDocXRS and VersaDoc Imaging Systems, Model 4000 and 5000
UVP	Chemi, BioChemi and OptiChemi Systems
Kodak	Image Station 2000MM
Fujifilm	LAS-3000 Imaging System
UVItec Ltd.	UVIchemi and UVIprochemi

III. APPLICATION NOTES

- 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.
- PicoTect™ Western Blot Chemiluminescent Substrate is extremely sensitive, requiring less sample and primary and secondary antibodies than other commercially available substrates, usually by a factor of at least 10-20.
- Primary antibodies purchased from Novus contain indications on the technical data sheet for use in Western Blot which are optimized for use with this substrate system.
- Blocking buffer formulations may need to be empirical tested to determine the appropriate conditions for each Western blot system. Determining the proper blocking buffer can help increase sensitivity and prevent non-specific 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 when the blocking buffer was not optimal for the new system. At Novus we use 5% Carnation nonfat dry milk in TBST (25 mM Tris-Cl, pH 8.0; 125 mM NaCl; 0.1% Tween 20).
- Avoid using milk as a blocking reagent when using avidin/biotin systems because milk contains variable amounts of endogenous biotin.
- The PicoTect™ Substrate Working Solution is stable for 8 h at RT. 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 typical laboratory lighting will not harm the Working Solution.
- Use a sufficient volume of all buffers and solutions to cover blot and ensure that it never becomes dry. Large blocking and wash buffer volumes may result in reduction of specific signal.
- For optimal results, use a shaking platform during incubation steps.
- Do not use sodium azide as a preservative for buffers. Sodium azide is an inhibitor of HRP and could interfere with this system.
- 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.

IV. NOVUS WESTERN BLOT PROTOCOL

1. Resolve equal amounts (about 10-50 μg/lane) of protein samples by SDS-PAGE and electro-blot using the Bio-Rad mini-gel transfer system (Bio-Rad Laboratories, Cambridge, MA) onto Immobilon P membranes (Millipore Corporation, MA), following manufacturers specifications.

2. Stain the blots with Amido black for 1 min and destained with 10% methanol plus 10% acetic acid. Amido black helps to monitor the efficiency of transfer without interfering with subsequent immuno-reaction. For easy labeling of the blot, allow it to air dry until the color fades.

3. Wet the blots in 100% methanol, rinse with TBST.

4. Block for 30-60 min with 5% Carnation nonfat dry milk in TBST (25 mM Tris-Cl, pH 8.0; 125 mM NaCl; 0.1% Tween 20) at RT on a shaking platform.

5. Incubate the blots with primary antibody in 1% milk/TBST overnight at 4°C. The primary antibody should be used at the concentration/dilution recommended on the technical data sheet, and diluted with 1% Carnation nonfat dry milk in TBST.

6. Wash with TBST, 5 x 5 min per wash.

7. Incubated with the proper secondary antibody-HRP (horse-radish peroxidase) conjugate for 60 minutes at 4°C (see the technical data sheet of each individual secondary antibody for recommended dilution). Dilute with 1% milk/TBST.

8. Wash the blot with TBST, 5 x 5 min per wash, to remove unbound HRP-conjugate.

9. Prepare the PicoTect™ Working Solution by mixing equal parts 1:1 of the PicoTect™ Substrate A and PicoTect™ Substrate B. Use 0.1 ml Working Solution per cm^2 of membrane or enough to ensure the entire blot is covered in solution. The PicoTect™ Working Solution is stable for 8 hours at RT.

Note: For best results keep the PicoTect™ Working Solution in an amber bottle and avoid prolonged exposure to any intense light. Typical laboratory lighting will not harm the Working Solution.

10. Incubate blot with the PicoTect™ Working Solution for 5 min at 4°C or RT.

11. Remove blot from PicoTect™ Working Solution and place it in a plastic membrane protector; a plastic sheet protector or plastic wrap may be used. Use an absorbent tissue to remove excess liquid and to carefully press out any bubbles from between the blot and surface of the membrane protector.

Note: Film must remain dry during exposure. For optimal results, perform the following precautions.

- *Make sure excess substrate is removed from the membrane and the membrane protector.*
- *Use gloves during the entire film-handling process.*
- *Never place a blot on developed film, as there may be chemicals on the film that will reduce signal.*

12. Turn off all lights except those appropriate for film exposure (e.g., a red safelight). Carefully place a piece of film on top of the wrapped membrane. A recommended first exposure time is 60 seconds. Exposure time may be varied to achieve optimal results. Enhanced or pre-flashed film is not necessary. We normally use Hyperfilm™ –ECL films (AMERSHAM LIFE SCIENCE INC.) and expose to the blots for 10 s, 1 min, 5 min, and 20 min to visualize the chemiluminescent signal corresponding to the specific antibody-antigen interaction.

Caution: Light emission is intense and any movement between the film and membrane can cause artifacts on the film.

Note: The exposure time may be varied to achieve optimal results. If the signal is too intense, reduce exposure time or optimize the system by decreasing the antigen and/or antibody concentrations. Light emission is most intense during the first 5-30 minutes after substrate incubation. Light emission will continue for several hours, but will decrease with time. Longer exposure times may be necessary as the blot ages. If using a storage phosphor imaging device (e.g., Bio-Rad's Molecular Imager® System) or a CCD Camera (e.g., Alpha-Innotech Corporation's ChemiImager™ System), longer exposure times may be necessary.

13. Blot may be stripped and reprobed if necessary.

V. REFERENCES

1. Eds Ole J. Bjerrum, Ph.D., M.D. and Niels H.H. Heegaard, M.D. CRC Handbook of Immunoblotting of Proteins: Volume 1 Technical Description. CRC Press, Inc.: Boca Raton, FL, 1988.

2. Kaufmann, S.H., Ewing CM, Shaper JH. The erasable Western blot. Anal. Biochem. 1987. 161:89-95.

3. Mattson, D.L. and Bellehumeur, T.G. Comparison of three chemiluminescent horseradish peroxidase substrates for immunoblotting. Anal. Biochem. 1996. 240:306-308.

4. Walker, G.R., Feather KD, Davis PD, Hines KK, SuperSignal™ CL-HRP: A new enhanced chemiluminescent substrate for the development of the horseradish peroxide label in Western blotting applications. J. of NIH Research 7:76. 1995.

VIII. TROUBLESHOOTING

Problem	Possible Cause	Solution
Reverse image on film (i.e., white bands with a black background)	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
Membrane has brown or yellow bands		
Blot glows in the darkroom		
Signal duration < 8 hours		
Weak or no signal	Too much HRP in the system depleted the substrate causing the signal to fade quickly	Dilute HRP-conjugate at least 10-fold
	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
	Too much HRP in the system	Dilute HRP-conjugate at least 10-fold
High background	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
Spots within the protein bands	Inefficient protein transfer	Optimize transfer procedure
	Unevenly hydrated membrane	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 0.2 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

Note: To test the activity of the system in the darkroom, prepare 1-2 ml of the SuperSignal® Substrate Working Solution in a clear test tube. With the lights turned off, add 1 ul undiluted HRP-conjugate to the Working Solution. The solution should immediately emit a blue light that will fade over the next several minutes.

If you require additional assistance, please contact Novus Technical Service:

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