WESTERN BLAST®

CHROMOGENIC BLOTTING AMPLIFICATION SYSTEM

NEL761001KT	NEL761A001KT
2,500 cm²	500 cm²

For Laboratory Use

CAUTION: A Research Chemical for Research Purposes Only

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I. INTRODUCTION

A. BLAST and Western Blotting

What is Western BLAST?

In Western blotting, complex mixtures of proteins are separated by electrophoresis and transferred to a membrane for subsequent immunological detection.

Western BLAST is a powerful technology from PerkinElmer, Inc., that enhances detection signals for chromogenic western blots at least 8-10 fold over conventional detection methods. It is easily integrated into standard protocols, provided that Horseradish Peroxidase (HRP) is in the system.

How does Western BLAST work?

Western BLAST uses proprietary CARD (catalyzed reporter deposition) technology for signal amplification in western blots. There is no change to western blotting protocols through protein transfer, blocking and incubation with HRP reagent. HRP catalyzes covalent bonding of biotin labels to adjacent proteins. The reaction is quick (less than 15 min) and results in the deposition of numerous biotin labels close to the enzyme. These labels can be detected by standard chromogenic techniques, with significant enhancement of the signal. Because the added labels are deposited adjacent to the enzyme site, there is minimal loss in resolution.

Chromogenic Detection Options

Western BLAST kits use an optimized CARD technology to deposit numerous biotin labels, as described above. These deposited biotins are detected with the following chromogenic detection options:

1. Using Western BLAST Kit Components: Chromogenic visualization is achieved using BLAST Streptavidin-Horseradish Peroxidase (SA-HRP) conjugate followed by the chromogen 4 CN *Plus*.

2. Alternative visualization options (requiring reagents not supplied):

a) BLAST SA-HRP followed by the chromogen DAB.

or

b) Streptavidin-Alkaline Phosphatase followed by BCIP/NBT.

Membrane Compatibility — BLAST has been successfully applied to nitrocellulose and PolyScreen PVDF, the preferred membranes for Western Blotting. (See Appendix 1 for PolyScreen PVDF membrane wetting protocol).

B. The Western BLAST kits

Western BLAST is compatible with a wide variety of standard immunological Western blotting protocols. However, HRP must be available for amplification to occur. Amplification is followed by standard chromogenic detection visualization techniques.

The Western BLAST kits for Western blotting contain the following components necessary for signal amplification:

Western BLAST Kit Components

NEL761A001KT	**500 cm^2**	**NEL761001KT**	**2,500 cm^2**
Reagent	**Amount**	**Reagent**	**Amount**
BLAST Streptavidin-HRP	68 µL	BLAST Streptavidin-HRP	320 µL
BLAST Blocking Reagent	1.0 gm	BLAST Blocking Reagent	5.0 gm
BLAST Amplification Diluent	17 mL	BLAST Amplification Diluent	80 mL
BLAST Control Protein	35 µL	BLAST Control Protein	35 µL
BLAST Biotin Reagent	17 mL	BLAST Biotin Reagent	80 mL
BLAST 4CN *Plus* Chromogenic Substrate	2.8 mL	BLAST 4CN *Plus* Chromogenic Substrate	12.8 mL
BLAST 4CN *Plus* Substrate Diluent	14 mL	BLAST 4CN *Plus* Substrate Diluent	64 mL

Storage and Stability	Upon receipt, the BLAST kit should be stored at 4°C. The Blocking reagent may be stored at room temperature if desired. The components in this kit are stable for a minimum of 6 months under proper storage conditions.

II. PROTOCOL

A. Introduction

Intended Use

FOR LABORATORY USE.

The intended use of this kit is to amplify signals generated by Horseradish Peroxidase. The reagents in this kit have been modified and optimized for use in membrane based procedures and are not meant for use with slide immunohistochemistry techniques or microtiter plates. Reagents for visualization with chromogens other than 4CN *Plus* must be purchased separately.

Safety Note

All reagents are classified as non-hazardous. We strongly recommend wearing disposable gloves and safety glasses while working. Thorough washing of hands after handling is also recommended. Do not eat, smoke, or drink in areas in which reagents are handled.

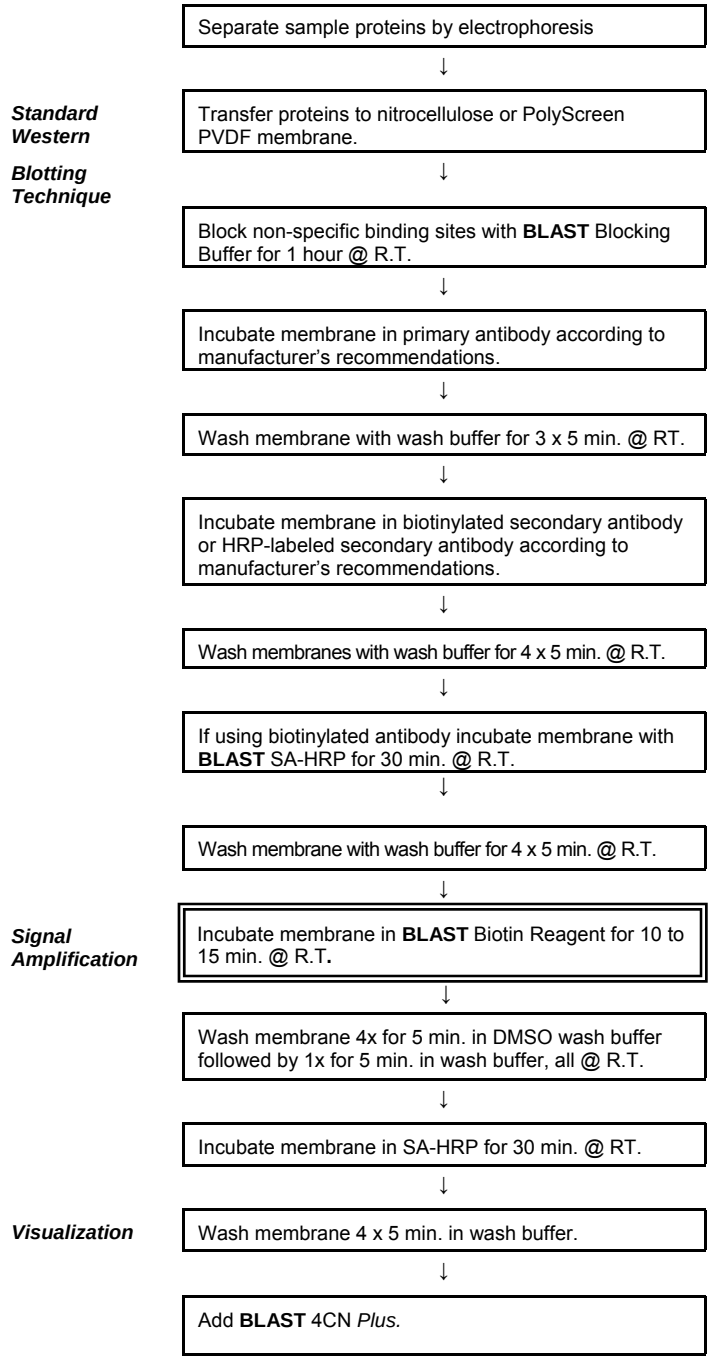
Additional Reagents

Phosphate Buffered Saline
Tween® 20
Dimethylsulfoxide (DMSO)
Bovine Serum Albumin (BSA)
PEG-8000
Distilled water

Additional Equipment

Standard laboratory glassware
Adjustable pipettors
Sterile pipets
Sterile pipet tips
Plastic boxes for incubation of membranes
Polypropylene tubes
Shaker
Plastic forceps
Powder-free gloves
Stirring hot plate

B. Overview of Protocol for Western BLAST



C. First Time Users

1. Use of Western BLAST Control Protein

The biotinylated control protein supplied in the kit should be used by first time users to evaluate the amplification technique.

Dilute the BLAST control protein in PBS (for nitrocellulose) or 40% ethanol/PBS (for PVDF) according to the following scheme:

Tube No.	Initial Volume	Add Volume of PBS or 40% Ethanol/ PBS
1	5 µL of control protein	20 µL
2	5 µL of tube # 1	20 µL
3	5 µL of tube # 2	20 µL
4	5 µL of tube # 3	20 µL
5	5 µL of tube #4	20 µL

Spot 1 µL of each dilution onto dry nitrocellulose or PolyScreen PVDF. Spot 2 separate membranes or membrane strips. One will be used for conventional detection and one used for amplification with Western BLAST. Both sets will be detected chromogenically with 4CN *Plus*.

Proceed to the Western BLAST Standard Protocol on p. 11. For conventional detection follow steps (2), (3), (4), (5) and (7) using SA-HRP at 1:1,000 in step (5). For amplified detection follow steps (2) through (7) using SA-HRP at 1:1,000 in step (5).

Results should show 1-2 more dots on the amplified strip.

2. Titration of Antibody Reagents

The high sensitivity achieved with Western BLAST detection may allow use of less primary and/or secondary antibody than that required for conventional chromogenic detection. Excess concentrations of antibodies in Western BLAST chromogenic detection can lead to high backgrounds or low signals. See Appendix 2 for suggested protocols for reagent titration.

Technical Support

If there are further questions regarding use of Western BLAST, please contact PerkinElmer Technical Support via email at techsupport@perkinelmer.com before proceeding.

D. Standard Protocol

1. Preparation of Buffers

The following buffers are required for Western BLAST amplification. (See Appendix 3 for detailed preparation and storage instructions and stability information).

DMSO Wash Buffer
Blocking Buffer
BSA Buffer
Wash Buffer (PBST)

2. Step by Step Protocol

Western Blotting

(1) Perform electrophoretic separation and transfer of proteins to appropriate membrane according to standard Western blotting protocol.

Blocking Step

(2) Incubate membrane with Western BLAST Blocking Buffer. Add 0.1 mL/ cm^2 and incubate at room temperature for 1 hour with gentle agitation.

NOTE: The Blocking Reagent supplied in the kit is optimal for use with the Western BLAST kit. Other blocking reagents may lead to increased background or reduced signal.

Suggested Antibody Incubation

(3) Drain off the BLAST Blocking Buffer and add primary antibody diluted in BLAST Blocking Buffer (or other buffer as appropriate).

Incubate the primary antibody preparation using the optimum concentration determined in Appendix 2. Follow manufacturer's instructions regarding incubation time and temperature requirements.

(4) Wash the membrane 4 x 5 min. in BLAST Wash Buffer at room temperature with gentle agitation.

Suggested Introduction of HRP

(5) Introduce HRP by one of the following options (adding volume of 0.1 mL/cm^2):

a. HRP labeled secondary antibody diluted in BSA Buffer. Incubate for 30-60 min. at room temperature with gentle agitation.

or

b. Biotin labeled secondary antibody diluted in BSA Buffer. Incubate for 30-60 min. at room temperature with gentle agitation. Wash membrane 4 x 5 min. in Wash Buffer at room temperature. Follow with SA-HRP diluted 1:1,000 in BSA Buffer. Incubate for 30-60 min. at room temperature with gentle agitation.

Amplification

(6) After HRP incubation, proceed with the following:

a. Wash the membrane 4 x 5 min. in Wash Buffer at room temperature with gentle agitation. Use at least 0.5 mL/cm^2 of membrane.

b. Immediately before use, prepare working dilution of BLAST Biotin Reagent by diluting 1:1 with BLAST Amplification Diluent. Add to membrane in volume of 0.0625 mL/cm^2. Incubate membranes at room temperature for 10-15 min. with gentle agitation. (This incubation step is facilitated by placing membrane in hybridization pouch. It is imperative to keep reagent evenly distributed over membrane).

c. Wash the membrane with DMSO Wash Buffer 4 x 5 min., followed by Wash Buffer for 1 x 5 min. at room temperature. Use at least 0.5 ml/cm^2 for each wash.

d. Dilute Western BLAST SA-HRP 1:1,000 in BSA Buffer. Add to membrane in a volume of 0.1 mL/cm^2. Incubate membranes at room temperature for 30 min. with gentle agitation.

e. Wash the membrane with Wash Buffer 4 x 5 min. at room temperature.

Chromogenic Visualization with BLAST 4CN *Plus*	(7) a.	Make 4CN *Plus* working dilution fresh before use. For 10 mL of chromogenic reagent, add 1 mL 4CN *Plus* Substrate Diluent to 9 mL distilled water. Add 0.2 mL 4CN *Plus* Chromogenic Substrate and mix well. The reagent may appear turbid.
	b.	Place the membrane in the prepared chromogenic reagent. Use at least 0.1mL/cm^2 and develop membrane in 4CN *Plus* Reagent for up to 30 min. Strong signals can appear faster, so development time should be monitored. Rinse membrane in water to stop the reaction.
Alternative Visualization	(8)	Alternatively chromogenic visualization can also be carried out with standard HRP catalyzed chromogenic substrates such as DAB (diaminobenzidine) and AEC (aminoethyl carbazole) or AP catalyzed substrates such as BCIP/ NBT (5-bromo-4-chloro-indolyl phosphate/nitro blue tetrazolium).
		NOTE: For AP catalyzed substrates, SA-AP is used in place of SA- HRP in step 6 (d).

III. TROUBLESHOOTING GUIDE

Problem	Remedy
Low Signal	· Perform titration of concentration of primary or secondary antibody and/or increase incubation time. · Lengthen incubation time of Biotin Reagent. · Multiple rounds of amplification may increase the signal.

Excess Signal	· Decrease concentration of primary and/or secondary antibody. · Decrease BLAST Biotin Reagent incubation time. · Decrease chromogenic substrate incubation time. · Decrease concentration of SA-enzyme conjugates.
High Background	· Decrease concentration of primary and/or secondary antibody or SA-HRP. · Decrease chromogenic substrate incubation time. · Increase number and/or length of washes. · Use only BLAST Blocking Buffer for blocking and antibody diluent.

IV. APPENDICES

APPENDIX 1: PolyScreen PVDF Membrane Wetting Protocol

Introduction

PolyScreen PVDF Membrane is extremely hydrophobic and will not wet in an aqueous solution unless the membrane is pre-wet with alcohol.

Protocol

Wet the membrane in 95% ethanol for at least one min. Soak the membrane until it changes from an opaque white to a uniform translucent gray.

Rinse the membrane in distilled water to wash off the alcohol for 2-3 min. If the membrane floats, gently push it into the water with plastic forceps until it wets.

Equilibrate the membrane in transfer buffer. Soak the membrane in the buffer for 10-15 min. to displace the water and any bubbles which may form.

NOTE: If the membrane dries (even partially) at any time during an experiment, you must wet it with alcohol and rinse with distilled water before proceeding.

APPENDIX 2: Titration of Antibody Reagents

Introduction

The high sensitivity achieved with Western BLAST detection sometimes requires the researcher to use less primary and/or secondary antibodies than that required for conventional chromogenic detection. Excess concentrations of antibodies in BLAST detection can lead to high background and/or low signals.

Titration

To achieve the maximum signal to noise ratio the primary and secondary antibodies should be optimized in a titration experiment. The following table gives an example of a typical titration experiment. The starting primary antibody dilution is 1:1,000 and the starting secondary antibody dilution is 1:1,000. The membrane samples are shown as #1 through #9.

The above titration allows the determination of the optimum concentration of the primary and secondary antibodies for BLAST chromogenic detection.

Buffer Formulations

PBS

Phosphate Buffered Saline, 10X (10X PBS)
To make 1 liter

$NaH_2PO_4 \cdot H_2O$	2.03 g
Na_2HPO_4	11.49 g
NaCl	85 g

The pH of the 10X solution is 6.7 to 6.9. The pH of the 1X solution should be 7.3 to 7.5. If not, adjust the 1X. Storage: Room temperature.

Alternatively, Dulbecco's Phosphate Buffered Saline without calcium chloride or magnesium chloride (available from commercial sources) may be used.

Western BLAST Wash Buffers

BLAST Wash Buffer (PBST)
Phosphate Buffered Saline, pH 7.4 (1X PBS)
0.05% TWEEN 20

	Primary Antibody Conc.		
Secondary Antibody Conc.	1:1,000	1:2,000	1:4,000
1:1,000	#1	#2	#3
1:2,000	#4	#5	#6
1:4,000	#7	#8	#9

DMSO Wash Buffer
1X PBS
0.05% TWEEN 20
20% DMSO

APPENDIX 3: Buffer Formulations

Western BLAST Blocking Buffer

1X PBS
0.05% TWEEN 20
1.0% BLAST Blocking Reagent
(supplied in kit)

Add Blocking Reagent slowly to buffer with vigorous stirring. Stir the solution at room temperature for at least 1 hour. Then, heat the Blocking Buffer gradually (up to 60°C) with continuous stirring to dissolve the Blocking Reagent. The solution should be milky white with no precipitate evident. Aliquot and store at -20°C for long term use.

NOTE: The Blocking Reagent supplied in this kit is optimal for use with the BLAST kit reagents provided. Other blocking reagents may lead to increased background and/or negligible signal amplification.

Western BLAST BSA Buffer

1X PBST
1.0% BSA
5.0% PEG-8000
Storage: 4°C for 1 month

APPENDIX 4: Simplified Western Blotting Protocol

Protocol

1. It is recommended that the transfer buffer be made up ahead of time and pre-cooled to 4°C. In this way, it will have a chance to degas before use. Bubbles in the transfer buffer will increase the chance of trapping air between the membrane and the gel. Air bubbles create points of high resistance, resulting in "bald spots" (i.e., areas of low-efficiency transfer and band distortion).

2. Cut the membrane slightly larger than the gel. If using a PolyScreen membrane, pre-wet with ethanol, then rinse with water. For nitrocellulose, just rinse with water. Be sure to wear gloves at all times when handling the membranes. Mark one side of the membrane for future reference.

3. Equilibrate both the membrane and the gel in transfer buffer for 15-20 min.

4. Wet two Scotch-Brite® pads and two pieces of filter paper (Whatman® 3MM cut to the size of the gel) in transfer buffer.

5. Prepare the "sandwich" as follows:

    - Put one piece of wet filter paper on a Scotch- Brite pad.

    - Place the equilibrated gel on top of the filter paper.

    - Place the membrane on top of the gel.

    - Place the second piece of wet filter paper over the membrane.

    - Be sure to remove any air bubbles trapped between the gel, membrane, and filter paper layers. This is easily done by rolling a clean pipet over the sandwich.

    - Complete the sandwich with the second Scotch-Brite pad.

6. Insert the sandwich into the transfer apparatus with the membrane positioned between the gel and the appropriate electrode. Most polypeptides are eluted from SDS-polyacrylamide gels as anions and therefore the membrane should usually be placed between the gel and the anode.

7. Fill the transfer apparatus with buffer. Pour the transfer buffer slowly to prevent bubble formation. Cool to 4°C and transfer at a constant current or voltage.

8. When the transfer is complete, remove the membranes and allow them to air dry at room temperature. Since dehydrated proteins bind more strongly to the membrane, this helps to prevent loss of target during subsequent washes.

V. COMPLEMENTARY PRODUCTS

HRP Conjugates	
Anti-rabbit IgG (goat) HRP	NEF812001EA
Anti-mouse IgG (goat) HRP	NEF822001EA
Anti-human IgG (goat)* HRP	NEF802001EA
Streptavidin HRP	NEL750001EA
Anti-DNP-HRP	FP1128
Antifluorescein-HRP	NEF710001EA
Biotin Conjugates	
Anti-rabbit IgG (goat) biotin	NEF813001EA
Anti-mouse IgG (goat) biotin	NEF823001EA
Anti-human IgG (goat) biotin	NEF803001EA
Labeled Streptavidin	
Streptavidin Fluorescein	NEL720001EA
Streptavidin Texas Red®	NEL721001EA
Streptavidin Coumarin	NEL722001EA
Streptavidin-HRP	NEL750001EA
Streptavidin-AP	NEL751001EA

PolyScreen PVDF Hybridization Transfer Membrane

26.5 cm x 3.75 m roll	NEF1002001PK
10 (20 x 20 cm) sheets	NEF1000001PK
50 (7 x 8.4 cm) sheets (for mini-gels)	NEF1003001PK

Protran® Nitrocellulose (0.2 ìm pore size)

30 cm x 3 m roll	NBA083C001EA
5 (15 x 15 cm) sheets	NBA083D001EA
5 (33 x 56 cm) sheets	NBA083G001EA

Protran® Nitrocellulose (0.45 ìm pore size)

15 cm x 3 m roll	NBA085A001EA
20 cm x 3 m roll	NBA085B001EA
30 cm x 3 m roll	NBA085C001EA
5 (15 x 15 cm) sheets	NBA085D001EA
5 (33 x 56 cm) sheets	NBA085G001EA

TSA Kits for Immunohistochemistry and In Situ Hybridization

TSA Fluorescein System	NEL701A001KT
TSA TMR System	NEL702001001KT
TSA Coumarin System	NEL703001KT
TSA Cyanine 3 System	NEL704A001KT
TSA Biotin System	NEL700A001KT

TSA Plus Kits for Immunohistochemistry and In Situ Hybridization

TSA Plus Fluorescein System	NEL741001KT
TSA Plus TMR System	NEL742001KT
TSA Plus Cyanine 3 System	NEL744001KT
TSA Plus Cyanine 5 System*	NEL745001KT
TSA Plus DNP (AP) System	NEL746B001KT
TSA Plus DNP (HRP) System	NEL747B001KT

Licensing

This product is covered by US patents 5,196,306, 5,583,001 and 5,731,158 and foreign equivalents owned by PerkinElmer Inc. It includes a license for research use only.

Notes

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