G-Biosciences ♦ 1-800-628-7730 ♦ 1-314-991-6034 ♦ technical@GBiosciences.com

A Geno Technology, Inc. (USA) brand name

femtoChromo™-AP

A Chromogenic Detection Kit for Western Blots

(Cat. # 786-379, 786-380, 786-381, 786-382, 786-383)

INTRODUCTION

The femtoChromo™ -AP kit is designed for the chromogenic detection and visualization of the proteins immobilized on transfer membranes (Western blots). This detection system uses affinity purified enzyme-linked (alkaline phosphatase; AP conjugated) antibodies and a sensitive chromogenic substrate BCIP-NBT. For immunodetection, when BCIP-NBT substrate is added to the transfer membrane containing the alkaline phosphatase labeled probes, a fine visible purple band develops at the site of AP activity. The supplied reagents are sufficient for approximately 4,000cm^2 of membrane when recommended volumes are used.

ITEM(S) SUPPLIED

Description	Cat # 786-379	Cat # 786-380	Cat # 786-381	Cat # 786-382	Cat # 786-383
BCIP-NBT Substrate	200ml	200ml	200ml	200ml	200ml
10X femto-TBST™	-	250ml	250ml	250ml	250ml
BLOT-QuickBlocker™	-	175g	175g	175g	175g
AP-goat α-mouse IgG [0.6mg/ml] (Cat # 786-R43)	-	-	1ml	-	1ml
AP-goat α-rabbit IgG [1mg/ml] (Cat # 786-R44)	-	-	-	1ml	1ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Store BLOT-QuickBlocker™ at ambient temperatures and all remaining kit components at 4°C. After reconstitution of the antibodies, they are stable for several weeks at 2-8°C as an undiluted liquid. For extended storage after reconstitution, add an equal volume of glycerol to make a final concentration of 50% glycerol (ACS grade or better) followed by storage at -20°C.

NOTE: *The concentration of antibody (and buffers) will decrease to one-half of the original after the addition of glycerol. Avoid repeated freeze/thaw cycles*

When used and stored properly the kit components are stable for one year.

ADDITIONAL ITEM(S) REQUIRED

Primary Antibody

ANTIBODY INFORMATION

Purification

The antibody is isolated from antisera by immunoaffinity chromatography using antigen coupled to sepharose beads.

Format

Lyophilized in a buffer containing 0.01M Sodium Phosphate, 0.25M NaCl, pH 7.6 with 15mg/ml BSA and 0.01% Thimerosal.

Reconstitution

Reconstitute with 1ml sterile distilled water.

Applications/Recommended Working Dilutions:

- EIA and Western blots: 1:5,000-1:100,000.
- Immunohistochemistry: 1:500-1:5,000.

NOTE: *Optimal working dilution of AP-conjugate for the specific application must be determined by the end user to obtain the best conditions. Working diluted solution should be prepared immediately before use and unused diluted solution should be discarded.*

Warning

Use of sodium azide, an inhibitor of phosphatases will substantially inhibit the enzyme activity of AP.

PREPARATION BEFORE USE

1X femto-TBST™

Dilute the 10X femto-TBST™ in deionized water by addining 1ml 10X femto-TBST™ to 9ml deionized water.

BLOT-QuickBlocker™

Prepare a 5% solution by dissolving 0.5g BLOT-QuickBlocker™ in 10ml 1X *femto*-TBST™.

Secondary Antibodies

Reconstitute with 1ml sterile distilled water. See storage conditions for storage of reconstituted antibodies.

PROTOCOL

Important Note: *Before starting the detection, mark the orientation of the protein samples on the transfer membrane. Never let the membrane dry at any step until the detection is complete. Use rotary shaker during all incubation / washing steps and use sterile water.*

1. After the electrophoresis transfer of the protein to an appropriate transfer membrane
2. Block the membrane by immersing in 5% solution of BLOT-QuickBlocker™ in for 1-2 hours at room temperature with gentle shaking. Discard the blocking solution.
3. Dilute primary antibody in 5% BLOT-QuickBlocker™ and add to the blot. Incubate for 1 hour at room temperature with gentle shaking.
   NOTE: *The correct antibody dilution must be empirically determined.*
4. Rinse the membrane twice with 1X *femto*-TBST wash buffer, then wash with 1X *femto*-TBST wash buffer 3 times, 10 minutes each at room temperature with gentle shaking.
5. Dilute the AP-conjugated secondary antibody in 5% BLOT-QuickBlocker™ and add to the blot. Incubate for 1 hour at room temperature with gentle shaking.
   NOTE: *The correct antibody dilution must be empirically determined.*
6. Rinse the membrane twice with 1X *femto* TBST wash buffer, then wash 3 times, 10 minutes each in 1X *femto* TBST wash buffer. Remove all wash buffer from the membrane before proceeding to the next step.
7. Apply BCIP-NBT substrate onto the membrane (approx 0.05ml/cm^2 of membrane). Allow the substrate to react at room temp with gentle shaking until suitable color intensity is observed (1-5 minutes or more).
8. Stop the color reaction by immersing the membrane in water and wash the membrane 2-3 times with water.
9. Allow the membrane to air dry, take a picture for a permanent record and store it sealed in a plastic pouch in the dark.

TROUBLESHOOTING

No Signal

1. Protein was not transferred completely from gel to the membrane or it has been over transferred and passed through the membrane.
2. Primary antibody was not of higher titer or specificity of alkaline phosphatase labeled secondary antibody was not appropriate for primary antibody.
3. Correct orientation of the membrane was not maintained throughout the procedure or the procedure was not followed properly or a step may have been omitted.

Weak Signal

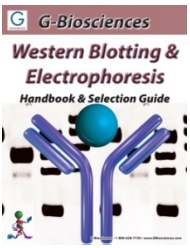
1. Antibody concentration was too low or incubation times were too brief.
2. Not enough protein was loaded onto the gel or the primary antibody has low affinity for the target protein.

Excessive Signal, Background or Non-Specific Signals

1. Antibody was not diluted sufficiently or incubation times are excessive (adjust dilution & incubation time).
2. Blocking or washing procedures are inadequate (follow the suggested protocol).
3. The amount antigenic protein loaded onto the gel is in excess.

RELATED PRODUCTS

Download our Western Blotting Handbook.



http://info.gbiosciences.com/complete-western-blot-handbook--selection-guide

For other related products, visit our website at www.GBiosciences.com or contact us.

Last saved: 5/14/2014 CMH

This page is intentionally left blank

www.GBiosciences.com