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A Geno Technology, Inc. (USA) brand name

femtoChromo™ HRP

A Chromogenic Detection Kit for Western Blots

(Cat. # 786-384, 786-385, 786-387, 786-388)

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INTRODUCTION

The femtoChromo™ HRP kit is designed for the chromogenic detection and visualization of the proteins immobilized on transfer membranes. The detection system uses affinity purified enzyme-linked (HRP conjugated) antibodies and an ultra-sensitive chromogenic substrate TMB (Tetramethylbenzidine). During the immunodetection process, when TMB substrate is added on to the membrane, the enzyme-linked (HRP labeled) antibodies initiate a color cascade that produces a band of visible precipitate on the transfer membrane. The reagents are sufficient for approximately 4,000cm^2 of membrane, when recommended volumes are used.

ITEM(S) SUPPLIED

Description	Cat. # 786-384	Cat. # 786-385	Cat. # 786-386	Cat. # 786-387	Cat. # 786-388
femtoChromo™ TMB Substrate	200ml	200ml	200ml	200ml	200ml
10X femtoTBST™ Buffer	-	250ml	250ml	250ml	250ml
BLOT-QuickBlocker™	-	175g	175g	175g	175g
Goat α-mouse IgG-HRP (Cat. # 786-R38)	-	-	2ml	-	2ml
Goat α-rabbit IgG-HRP (Cat. # 786-R39)	-	-	-	2ml	2ml

STORAGE CONDITIONS

The kit is shipped at ambient temperature. Kit components should be stored refrigerated (4°C), except BLOT-QuickBlocker™ at room temperature and antibodies at -20°C. When used and stored properly the kit components are stable for one year.

ADDITIONAL ITEMS REQUIRED

1. Primary Antibody.
2. Stripping and Reprobing Buffer *(Western ReProbe, Cat. # 786-119)*.

PROTOCOL

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, block the membrane by immersing in 5% solution of BLOT-QuickBlocker™ in 1X *femto*TBST™ buffer, (dilute 10X *femto*TBST™ 10 fold with water). Block for 1-2 hours at room temperature with gentle shaking.
2. Dilute primary antibody in freshly prepared blocking buffer. Proper antibody dilution must be empirically determined (See Application Notes). Incubate the blot in the diluted primary antibody for 1 hour at room temperature with gentle shaking.
3. Rinse the membrane twice with 1X *femto*TBST™ wash buffer, then wash with 1X *femtoTBST™* wash buffer 3 times, 10 minutes each at room temperature with gentle shaking.
4. Dilute the HRP-conjugated secondary antibody in the freshly prepared blocking buffer. Proper antibody dilution must be empirically determined (See Application Notes). Incubate the blot in the diluted secondary antibody for 1 hour at room temperature with gentle shaking.
5. Rinse the membrane twice with 1X *femtoTBST™* wash buffer, then wash 3 times, 10 minutes each in 1X *femtoTBST™* wash buffer. Remove all the wash buffer from the membrane before proceeding to the next step

   OPTIONAL*: Air dry the membrane on a piece of Whatmann paper to remove excess liquid.*
6. Apply 0.05ml *femto*Chromo™ TMB substrate for every cm^2 of membrane. For example for an 8x10cm membrane apply 4ml *femto*Chromo™ TMB substrate.
7. Allow the substrate to react at room temperature with gentle shaking until suitable color intensity is observed (approx 1-5 minutes).
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.

APPLICATION NOTES

Primary & Secondary Antibody Dilution

Proper antibody dilution must be empirically determined.
For primary, usually it is 1:100-1:1000 for polyclonal antibodies, 1:10-1:100 for hybridoma supernatants and >1:1000 for monoclonal antibodies.
For HRP conjugated secondary antibodies, usually it is 1:500-1:2000. It may be necessary to perform a serial dilution of primary & secondary antibodies through a dot blot to determine the optimal working concentration.

TROUBLE SHOOTING

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 peroxidase 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

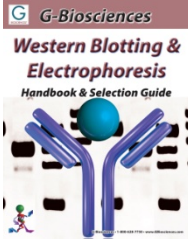
1. Antibody concentration was too low or incubation times were too brief.
2. Not enough protein was loaded onto the gel or the primary antibody has low affinity for the target protein.

Excessive Signal, Background or Non-Specific Signal

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 of 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.

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