picoLUCENT™ PLUS-HRP

G-Biosciences

Abstract

Chemiluminescence Detection System for Horseradish Peroxidase

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Guidelines

INTRODUCTION

picoLUCENT™ PLUS-HRP kit is based on our ultra sensitive Luminol substrate that produces chemiluminescence upon reaction with horseradish peroxidase. The chemiluminescence light emission can be recorded by a short exposure to autoradiography films.

picoLUCENT™ PLUS-HRP reagents are available in five sizes suitable for 5, 25, 50, 125 and 250 blots as each 4ml of working solution is suitable for 1 mini blot (8 x 7.5cm)

In addition, picoLUCENT™ PLUS-HRP is also supplied in a kit format, containing our non- animal protein blocking agent (NAP-BLOCKER™) and wash buffer (femto-TBST™) The picoLUCENT™ PLUS-HRP kits allow detection of low picogram levels (10-12) of antigens with low noise (signal/background) ratio. The kit reagents are sufficient for 25 mini blots or 1,500cm2 of PVDF or nitrocellulose membrane.

ITEM(S) SUPPLIED

Cat. #	Size*	NAP-BLOCKER™ [2X]	femto TBST [10X]	Luminol Solution	Peroxide Solution
786-09T	5	-	-	10ml	10ml
786-002	25	-	-	50ml	50ml
786-09	25	250ml	250ml	50ml	50ml
786-165	50	-	-	100ml	100ml
786-264	125	-	-	250ml	250ml
786-424	250	-	-	500ml	500ml

* For mini blots with average size of 8 x 7.5cm or 60cm$^{2.}$

STORAGE CONDITIONS

Shipped at ambient temperature. Upon arrival, store the kit components at 4°C, protected from light. When stored and used properly, the kit is stable for 1 year.

ITEMS NOT SUPPLIED

• Primary antibody

• Secondary antibodies, HRP-conjugates.

PREPARATION BEFORE USE

1. **Preparation of Working Detection Solution:** Allow the solutions to warm to room temperature before use. For each mini blot membrane (~60cm^2), mix 2.0ml of picoLUCENT™ Luminol Solution-A and 2.0ml picoLUCENT™ Peroxide Solution-B.

2. **Preparation of 1X femto-TBST:** Dilute the appropriate volume of supplied 10X femto-TBST to 1X with DI Water (e.g. Take 10ml of 10X femto-TBST and add 90ml DI Water to make it 1X).

3. **Preparation of 1X NAP-BLOCKER™:** Use **aseptic techniques** for handling NAP- BLOCKER™ . Allow the supplied 2X NAP-BLOCKER™ bottle to come to room temperature and then gently shake to mix. Dilute the appropriate volume of 2X NAP-BLOCKER™ 1:1 with 1X femto-TBST (e.g. Take 10ml of 2X NAP-BLOCKER™ and add 10ml of 1X femto-TBST).

PROTOCOL FOR REAGENT ONLY

1. Prepare the Working Detection Solution as described above.
2. Block and probe your membrane with appropriate blocking agents and antibodies as per your regular protocols.
3. Following the final wash steps, add 4ml Working Detection Solution for a mini blot and incubate for 3-5 minute at room temperature with gentle shaking.
4. Drain the detection reagent and wrap the membrane in saran wrap or clear plastic wrap and expose to an autoradiography film.

***NOTE**: Do NOT wash or rinse the membrane after addition/removal of the working detection solution.*

REDEVELOPING THE MEMBRANE

The membrane can be redeveloped within a day or two, provided that the detection reagents are removed from the membrane within 30-60 minutes of the first developing procedure. After each developing procedure, wash the membrane with 50ml TBS with Tween-20. Keep the membrane moist and at 4°C. Redevelop the membrane according to the protocol above and expose the autoradiography film.

TROUBLESHOOTING

Uniform High Background

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in a high background.
Interference from incompatible blocking agent	Investigate a different blocking agent, such as non animal protein blocking agents.
Antibodies cross-react with proteins present in blocking agents	Investigate a different blocking agent, such as non animal protein blocking agents. Avoid milk based blocking agents when probing with avidin/biotin systems. Milk contains biotin.
Non-specific sites insufficiently blocked	Optimize the blocking buffer and conditions, including amount and type of blocking protein (agent) and length and temperature of blocking step. Add Tween® 20 to the blocking agent, if detergent is not already present. Final concentration of 0.05%. Incubate with antibodies in blocking agent containing 0.05% Tween® 20.
Washing steps insufficient	Increase volume and length of wash steps. Use wash buffers with Tween® 20, such as out femto- TBST™ or femto-TBST™ Wash Buffers.
Membrane exposed too long to film	Reduce the exposure time.
Membrane issues	Membranes not wetted correctly, check manufacturer's instructions. Membrane inadvertently dried out during procedure. Use orbital shaking or rocking with all incubation steps. Handle membrane carefully, do not touch with exposed skin.
Bacterial or other contamination	Prepare fresh buffers.

Blotchy or Speckled High Background

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in a high background.
Interference from incompatible blocking agent	Investigate a different blocking agent, such as non animal protein blocking agents.
HRP conjugate may have aggregated	Filter through a 0.2µm filter. Use new conjugate.
Antibodies cross-react with proteins present in blocking agents	Investigate a different blocking agent, such as non animal protein blocking agents. Avoid milk based blocking agents when probing with avidin/biotin systems. Milk contains biotin.
Non-specific sites insufficiently blocked	Optimize the blocking buffer and conditions, including amount and type of blocking protein (agent) and length and temperature of blocking step. Add Tween® 20 to the blocking agent, if detergent is not already present. Final concentration of 0.05%.Incubate with antibodies in blocking agent containing 0.05% Tween® 20.
Washing steps insufficient	Increase volume and length of wash steps. Use wash buffers with Tween® 20, such as out femto- TBST™ or femto-TBST™ Wash Buffers.
Membrane exposed too long to film	Reduce the exposure time.
Membrane issues	Membranes not wetted correctly, check manufacturer's instructions. Membrane inadvertently dried out during procedure. Use orbital shaking or rocking with all incubation steps. Handle membrane carefully, do not touch with exposed skin.
Bacterial or other contamination	Prepare fresh buffers.
Dirty equipment	Ensure all equipment is free of contaminants. Ensure no residual gel pieces are present on the membrane.

Weak or No Signal

Suggested Cause	Resolution/ Precaution
Improper transfer of proteins to membrane	Ensure correct protein transfer by staining the membrane with a suitable, reversible membrane stain. We recommend Swift™ Membrane Stain (Cat. No. 786-677). For poor transfer of high molecular weight proteins, use our High Molecular Weight Transfer Buffer (Cat. No. 786-423). Ensure the transfer sandwich and apparatus is assembled correctly and electrodes are correctly orientated. Avoid over heating during transfer.
Poor binding of protein to membrane	Add 20% methanol to transfer buffer for improved binding. For low molecular weight proteins, reduce transfer time or use a membrane with a smaller pore size to prevent proteins passing through.
Concentration of antibody too high	Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in the signal generating too quickly and fading away before development.

Concentration of antibody too low	Increase antibody concentration to overcome possible poor affinities.
Antigen levels too low	Load more proteins on the initial gel.
Blocking agent binds antigen	Optimize blocking agent type and concentration.
HRP activity inhibited by sodium azide	Avoid using buffers that use sodium azide as a preservative.
Exposure time too short	Extend the film exposure time.
Detection substrate inactive	Ensure the substrate(s) shelf life has not expired. Cross reaction between the 2/3 component systems may have occurred. Check for activity by preparing substrate and, in a dark room, add a small amount of conjugate. If active a blue light should appear.
Excessive stripping	If the membrane has been stripped, antigen sites may have been destroyed. Use mild stripping conditions, we recommend Western ReProbe™ (Cat. No. 786-119). Limited the number of times a membrane is reprobed.

Non-Specific Bands

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in non-specific binding.
Presence of SDS	Thoroughly wash blot after transfer. Do not use SDS in development steps.

Diffuse Bands

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in non-specific binding.
Protein concentration too high	Reduce the amount of protein loaded

Ghost/ Hollow Bands or Brown/Yellow Bands on Membrane

Suggested Cause	Resolution/ Precaution
Concentration of antibody too high	Reduce the concentration of primary and/ or secondary antibodies as high concentration can result in rapid consumption of the substrate.

Blank Areas

Suggested Cause	Resolution/ Precaution
Incomplete transfer of proteins	Ensure correct protein transfer by staining the membrane with a suitable, reversible membrane stain. We recommend Swift™ Membrane Stain (Cat. No. 786-677). Ensure the transfer sandwich and apparatus is assembled correctly and electrodes are correctly orientated. Avoid over heating during transfer.

STRIPPING AND RE-PROBING MEMBRANE

The developed membrane can be stripped and re-probed with any other antibody by using a suitable stripping buffer. G-Biosciences Western-Re-Probe™ Buffer (5X) is recommended for stripping and re-probing procedures.

CITATIONS

1. Maruscak, A., et al (2008) Am J Physiol. Lung Cell Mol. Physiol., 294: L974-L983

Before start

1. **Preparation of Working Detection Solution:** Allow the solutions to warm to room temperature before use. For each mini blot membrane (~60cm^2), mix 2.0ml of picoLUCENT™ Luminol Solution-A and 2.0ml picoLUCENT™ Peroxide Solution-B.

2. **Preparation of 1X femto-TBST:** Dilute the appropriate volume of supplied 10X femto-TBST to 1X

with DI Water (e.g. Take 10ml of 10X femto-TBST and add 90ml DI Water to make it 1X).

3. **Preparation of 1X NAP-BLOCKER™:** Use **aseptic techniques** for handling NAP- BLOCKER™ . Allow the supplied 2X NAP-BLOCKER™ bottle to come to room temperature and then gently shake to mix. Dilute the appropriate volume of 2X NAP-BLOCKER™ 1:1 with 1X femto-TBST (e.g. Take 10ml of 2X NAP-BLOCKER™ and add 10ml of 1X femto-TBST).

Materials

picoLUCENT™ PLUS-HRP 786-09 by G-Biosciences

Protocol

Blocking

Step 1.

After the electrophoretic transfer of the protein to an appropriate membrane (e.g. PVDF or Nitrocellulose), block the membrane by immersing in 10ml 1X NAP-BLOCKER™.

Blocking

Step 2.

Incubate the blot (membrane) in the blocking buffer for a minimum of 60 minutes at room temperature with gentle shaking on an orbital shaker.

DURATION
01:00:00

Primary Antibody Treatment

Step 3.

Dilute the primary antibody in an appropriate volume (≤5ml) of 1X NAP-BLOCKER™.

Primary Antibody Treatment

Step 4.

Incubate the membrane in the diluted primary antibody for 1-2 hours at room temperature, with gentle shaking.

DURATION
01:00:00

NOTES
Colin Heath 22 Jun 2016

NOTE: Determine the optimal dilution of the primary antibody in separate experiments or follow

the manufacturer’s instructions.

Washing

Step 5.

Rinse the membrane with 10ml 1X femto-TBST.

Washing

Step 6.

Wash with 10ml 1X femto-TBST buffer for 10 minutes at room temperature with gentle shaking. (wash 1/3)

DURATION
00:10:00

Washing

Step 7.

Wash with 10ml 1X femto-TBST buffer for 10 minutes at room temperature with gentle shaking. (wash 2/3)

DURATION
00:10:00

Washing

Step 8.

Wash with 10ml 1X femto-TBST buffer for 10 minutes at room temperature with gentle shaking. (wash 3/3)

DURATION
00:10:00

Secondary Antibody Treatment

Step 9.

Dilute the HRP-conjugated secondary antibody in an appropriate volume (≤5ml) of 1X NAP-BLOCKER™ at a 1:5,000 to 1:100,000 dilution.

NOTES
Colin Heath 22 Jun 2016

NOTE: Determine the optimal dilution of the secondary antibody in separate experiments.

Secondary Antibody Treatment

Step 10.

Incubate the membrane in the diluted secondary antibody for 1-2 hours at room temperature with gentle shaking.

DURATION

01:00:00

Washing

Step 11.

Rinse the membrane with 10ml 1X femto-TBST.

Washing

Step 12.

Wash with 10ml 1X femto-TBST buffer for 10 minutes at room temperature with gentle shaking. (wash 1/3)

DURATION
00:10:00

Washing

Step 13.

Wash with 10ml 1X femto-TBST buffer for 10 minutes at room temperature with gentle shaking. (wash 2/3)

DURATION
00:10:00

Washing

Step 14.

Wash with 10ml 1X femto-TBST buffer for 10 minutes at room temperature with gentle shaking. (wash 3/3)

DURATION
00:10:00

Chemiluminescence Reaction

Step 15.

Incubate the membrane in the 4ml Working Detection Solution for 3-5 minute at room temperature with gentle shaking.

DURATION
00:03:00

NOTES
Colin Heath 10 Jan 2017

NOTE: Do NOT wash or rinse the membrane after addition/removal of the working detection solution.

Step 16.

Drain the detection reagent and wrap the membrane in saran wrap or clear plastic wrap and expose to an autoradiography film.

NOTES

Colin Heath 22 Jun 2016

NOTE: Do NOT wash or rinse the membrane after addition/removal of the working detection solution.