KPL LumiGLO Ultra™ Western Blotting Substrate

Catalog No.	Size
5430-0039 (54-51-00)	50 mL

DESCRIPTION

KPL LumiGLO Ultra Western Blotting Substrate contains an acridane-based chemiluminescent substrate designed for use with peroxidase-labeled (HRP) reporter molecules. KPL LumiGLO Ultra offers several improvements over LumiGLO® or similar competitive substrates. These include a 20-fold sensitivity boost and optimization for protein detection where low abundance or small sample size impact detection. KPL LumiGLO Ultra Western Blotting Substrate is provided as a stable two-component solution, to be prepared in a 1:1 ratio. Results can be obtained on a chemiluminescent imager to provide a permanent record. KPL LumiGLO Ultra produces light that is greater in intensity than many traditional chemiluminescent systems.

CONTENTS

1 x 25 mL KPL LumiGLO Ultra Chemiluminescent Substrate Solution A
1 x 25 mL KPL LumiGLO Ultra Chemiluminescent Substrate Solution B
Individual reagents are not sold separately.
Reagents are provided for detection of 1000 cm^2 of membrane.

STORAGE/STABILITY

Store all components at 2–8°C. Stable for a minimum of one year from date of receipt when stored under proper conditions. Prepared KPL LumiGLO Ultra working solution is stable for one week at room temperature when protected from light, or 1 month at 2-8°C. Substrate should be used in subdued light. Exposure to direct light will cause elevated background.

PRODUCT SAFETY AND HANDLING

See SDS (Safety Data Sheet) for this product.

KPL LUMIGLO ULTRA WESTERN BLOTTING SUBSTRATE USER'S GUIDE

- KPL LumiGLO Ultra can be used with nitrocellulose and PVDF membranes. For maximum signal to noise, nitrocellulose is recommended.
- The KPL LumiGLO Ultra working solution should be protected from light after preparation and warmed to room temperature prior to use.
- For maximum signal, expose membrane to imager immediately after incubation with KPL LumiGLO Ultra. For most applications, exposures of 10 minutes or less produce sufficient sensitivity.
- KPL LumiGLO Ultra is an extremely sensitive substrate. Insufficient washing of membranes or contamination of substrate with HRP will result in non-specific background.
- Because of KPL LumiGLO Ultra's high sensitivity, it is imperative that the HRP conjugate be titrated to give optimal signal to noise.
- The primary antibody should be purified for best results.
- Because of its high light intensity, most images may be captured within 10 minutes, making multiple exposures easy to obtain.

APPLICATIONS

KPL LumiGLO Ultra Chemiluminescent Substrate has been optimized for Western blotting and dot blotting applications. The following is a recommended procedure for Western blot detection.

Suggested Reagents/Equipment Not Included

1. Primary antibody (Purified)
2. HRP-labeled secondary antibody
3. Nitrocellulose or PVDF membrane
4. Blocking Solution (See RELATED PRODUCTS)
5. Wash Solution (See RELATED PRODUCTS)
6. CCD Imager
7. Platform shaker or rocker
8. Incubation trays or tubes

CONJUGATE OPTIMIZATION PRIOR TO DETECTION

- Before beginning the assay, it is imperative that the optimal conjugate dilution be determined for the assay.
- The use of highly sensitive chemiluminescent substrates on Western blots can cause high background if the conjugate concentration is not optimized. Each lot of conjugate will need optimization as slight differences in activity can result in major differences in background.
- Recommended conjugate dilutions should be tested at a range from 1/25,000 – 1/250,000 of a 1.0 mg/mL stock.

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WESTERN BLOT DETECTIONAT A GLANCE

Total Time: 4 hours

Polyacrylamide Gel Electrophoresis

⇓

Immobilize Protein on Membrane

⇓

Block Membrane

1 hour or overnight

⇓

Incubate Primary Antibody

30 minutes - 1 hour

⇓

Wash Membrane

3 x 5 minutes per wash

⇓

Incubate Conjugate

30 minutes - 1 hour

⇓

Wash Membrane

1X Wash Solution, 3 x 5 minutes per wash

1X Wash Solution, 1 x 15 minutes

Reagent Quality Water, 1 x 5 minutes

⇓

Incubate with KPL LumiGLO Ultra

1 minute

⇓

Expose to Imager

10 SECONDS - 10 MINUTES

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DETECTION OF WESTERN BLOTS

There are many protocols available for the detection of Western blots. For optimal signal to noise and sensitivity, the following protocol and reagents are recommended.

STEPS	CRITICAL POINTS
1. Block the membrane by immersing in 1X KPL SignaLOCK Blocking Solution using a minimum of 0.2 mL/cm2 of membrane. Block at room temperature for 1 hour with gentle rocking or shaking, or stationary at 2–8°C overnight. If blocking overnight, container should be covered.	*Dilute KPL SignaLOCK Blocking Solution 1:5 in reagent quality water. Make sure to use a container of proper size that allows the block solution to freely float over the membrane.*
2. Remove blocking solution. Incubate membrane with purified primary antibody diluted 1:1,000 – 1:10,000 from a 1 mg/mL stock for 30 minutes - 1 hour. This antibody should be diluted in fresh blocking solution.	*It is recommended that serial dilutions of the primary antibody are tested on a dot blot to determine the optimal working dilution.*
3. Remove primary antibody in block solution. Wash the membrane in a generous amount of 1X Wash Solution (at least 25 mL for a 100 cm^2 membrane). Wash membrane 3 times for 5 minutes per wash with shaking.	*Dilute 20X KPL Wash Solution 1:20 in reagent quality water. This solution will provide optimal signal to noise.*
4. Dilute appropriate HRP-labeled secondary antibody 1/25,000 – 1/250,000 of a 1.0 mg/mL stock in freshly prepared 1X KPL SignaLOCK Blocking Solution using a minimum of 0.2 mL/cm^2 of membrane.	*The optimal dilution may vary for different lots of conjugate. It is imperative that you titrate the conjugate to determine the optimal working dilution. These dilutions hold true for either AP or HRP.*
5. Incubate blot with diluted conjugate for 30 minutes - 1 hour at room temperature with shaking.	
6. Remove KPL LumiGLO Ultra from refrigeration. Mix equal parts KPL LumiGLO Ultra Solution A with Solution B. Protect mixed solution from light until use. Avoid vigorous agitation. Allow mixed working solution to sit for at least 2 minutes prior to use.	*Prepare KPL LumiGLO Ultra working solution in advance to allow it to warm to room temperature prior to use. Minimize exposure to light. 0.05 mL/cm^2 is the minimum volume necessary to cover the membrane.*
7. Wash the membrane in a generous amount of 1X KPL Wash Solution (at least 25 mL for a 100 cm^2 membrane). Wash membrane 3 times with five minutes per wash, then once for 15 minutes. Perform all washes with shaking.	
8. Rinse the membrane once for 5 minutes in reagent quality water.	
9. Pour off the water from the blot and place the membrane on a sheet protector or a dry tray. Membrane should be slightly damp.	
10. Pipette 0.05 mL/cm^2 of the previously prepared KPL LumiGLO Ultra onto the membrane. Incubate ***without*** rocking for 1 minute.	*Example: for a 10 x 10 cm blot, use 5 mL of KPL LumiGLO Ultra. The surface tension of the substrate will keep it on the surface of the membrane.*
11. Blot the membrane thoroughly dry with filter paper.	*Excess KPL LumiGLO Ultra on the blot will contribute to background. Follow the manufacturer's recommendations regarding the set up and operation of the imager.*
12. Lift the membrane with clean forceps and place in the imaging system. Collect images for 10 seconds – 10 minutes. Peak signal intensity for KPL LumiGLO Ultra is at ~ 10 minutes.	

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TROUBLESHOOTING

Problem 1: No Signal

Possible Cause	Corrective Measure
Inactive HRP conjugate	Verify conjugate activity by mixing 10 µL of diluted conjugate with 1 mL of substrate (in a dark room, the substrate should glow).
No binding of conjugate to the primary antibody.	Confirm correct specificity of the conjugate for the primary antibody; *i.e*. no anti-rabbit HRP with a mouse primary antibody.
No transfer of target to membrane	Use a protein stain (ex. –Ponceau S) on unblocked membrane to verify attachment of target protein or use a pre-stained protein marker to monitor transfer.
Detection of nonblotted side of membrane	Ensure correct orientation of the membrane during the assay and image capture.
Inhibition of horseradish peroxidase	Ensure buffers do not contain sodium azide; azide will inhibit horseradish peroxidase activity.
Missed step in procedure	Review procedure to ensure all steps were followed.

Problem 2: Weak Signal

Possible Cause	Corrective Measure
Insufficient amount of antibody	Optimize antibody concentrations. Affinity of the primary antibody may change after proteins are denatured through SDS-PAGE.
Insufficient protein loaded or transferred	Increase the amount of protein loaded onto the gel.
Insufficient incubation of primary antibody to target	Increase the incubation times for weak primary antibodies.
Insufficient exposure time	Increase the time of exposure.
Excessive washing beyond recommended procedure	Follow the procedure as written.

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Problem 3: Excessive signal, nonspecific bands or general background

Possible Cause	Corrective Measure
Overexposed image	Expose the membrane for a shorter period of time.
Insufficient blocking or washing	Increase blocking and washing time or increase number of washes.
Excessive antibody used for detection	Optimize conjugate concentration. Reduce antibody concentrations; optimal conjugate dilution should be 1/25,000 – 1/250,000 of a 1.0 mg/mL stock. OR Decrease the amount of primary antibody.
Excessive protein loaded on the gel	Decrease the amount of protein loaded onto the gel.
Endogenous peroxidase in the sample	Test by incubating the blocked membrane in KPL LumiGLO Ultra (without antibodies). After film exposure, if signal is obtained, blocking reagents such as 3% H_2O_2 in 100% MeOH may be required to remove the endogenous activity.

Problem 4: Poorly Defined or "Fuzzy" Bands or Dots

Possible Cause	Corrective Measure
Poor transfer of protein to membrane	Follow manufacturer's recommended procedure or contact the manufacturer for additional support regarding the blotting apparatus.
Excessive LumiGLO Ultra on membrane	Remove excess KPL LumiGLO Ultra before exposure of the membrane. Blot thoroughly.
Inadequate handling of membranes	Certain membranes require special handling. Check with the membrane vendor for correct procedures.

Stripping and Reprobing a Western Blot

This protocol is adapted from Kaufmann, *et al*[8]. After performing protein transfer, detection with KPL LumiGLO Ultra and imager analysis, membranes may be stripped and reprobed with new primary and secondary antibodies.

1. Strip antibodies by incubating blot for 30 - 90 minutes at 70°C in erasure buffer: 2% SDS (w/v), 62.5 mM Tris-HCl (pH 6.8 at 20°C), 100 mM β-mercaptoethanol.
2. Wash 2 times, for 10 minutes each, in TBS: 10 mM Tris-HCl (pH 7.4 at 20°C), 150 mM NaCl.
3. Block for 2.5 hours in 1X KPL SignaLOCK Blocking Solution.
4. Repeat detection procedure.

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REFERENCES

1. Kricka, L. J. (1991). Chemiluminescent and Bioluminescent Techniques. *Clin. Chem*. 37(9): 1472 - 1481.
2. Knect, D.A. and R.L. Dimond (1984). Visualization of Antigenic Proteins on Western Blots. *Anal. Biochem.* 136: 180 - 184.
3. Isacsson, V. and G. Wettermark (1974). Chemiluminescence in Analytical Chemistry. *Anal. Chim. Acta*. 68: 339 - 362.
4. Towbin, H., T. Staehelin and J. Gordon (1979). Electrophoretic Transfer of Proteins From Polyacrylamide Gels to Nitrocellulose Sheets:Procedure and Some Applications. *Proc. Natl. Acad. Sci. USA*. 76: 4350 - 4354.
5. Burnette, W.N. (1980). "Western Blotting": Electrophoretic Transfer of Proteins From Sodium Dodecyl Sulfate-Polyacrylamide Gels to Unmodified Nitrocellulose or Nitrocellulose Sheets. *Anal. Biochem.* 112: 195 - 203.
6. Reinhart, M.P. and D. Malamud (1982). Protein Transfer From Isoelectric Focusing Gels:The Native Blot. *Anal. Biochem.* 123: 229 - 235.
7. Gooderham, K (1983). *Protein Blotting*. *In* J. Walker and W. Gaastra (eds.), Techniques in Molecular Biology. Croom Helm Ltd. Publishers, London.
8. Kaufmann, Ewing and Shaper (1987). The Erasable Western Blot. *Anal. Biochem*. 161, 89 - 95.

RELATED PRODUCTS	CAT. NO.
KPL SignaLOCK™ HRP ChemiWestern Kit (Imager)	5410-0011 (54-54-00)
KPL 5X SignaLOCK Blocking Solution	5440-0001 (50-58-00)
KPL 20X Wash Solution	5150-0008 (50-63-00)
KPL Anti-Human IgG (H+L) Antibody, Peroxidase labeled, 1.0 mg	5220-0330 (074-1006)
KPL Anti-Rabbit IgG (H+L) Antibody, Peroxidase labeled, 1.0 mg	5220-0336 (074-1506)
KPL Anti-Mouse IgG (H+L) Antibody, HSA and Peroxidase labeled, 1.0 mg	5220-0341 (074-1806)

The product listed herein is for research use only and is not intended for use in human or clinical diagnosis.