KPL LumiGLO Reserve™ Chemiluminescent Substrate

Catalog No.	Size
5430-0050 (54-71-01)	600 cm^2
5430-0051 (54-71-02)	1000 cm^2
5430-0049 (54-71-00)	2400 cm^2

DESCRIPTION

KPL LumiGLO Reserve Chemiluminescent Substrate contains a luminol-based chemiluminescent substrate designed for use with peroxidase-labeled (HRP) reporter molecules. KPL LumiGLO Reserve offers improvements in the way of signal intensity greater than 20-fold more sensitive than KPL LumiGLO® and other competitive substrates. These products are specifically designed for the detection of proteins that are either in low abundance or are from samples that are precious and, therefore, desired to be conserved.

KPL LumiGLO Reserve Chemiluminescent Substrate is provided as a stable two-component solution, to be prepared in a 1:2 ratio. This combination provides rapid and accurate identification of proteins that are of low abundance and potentially limited availability. Given the increased sensitivity, less target may be required.

Three sizes are available. A concentrated KPL Wash Solution is also provided for added convenience. Results can be obtained on X-ray film or a chemiluminescent imager to provide a permanent record. In fact, this kit provides significant light output that is more readily visualized by chemiluminescent imagers than many traditional chemiluminescent systems.

CONTENTS

5430-0049 (54-71-00) for 2400 cm^2, contains:
1 x 40 mL KPL LumiGLO Reserve Substrate Solution A
1 x 80 mL KPL LumiGLO Reserve Substrate Solution B
2 x 200 mL KPL Wash Solution Concentrate (20X)

5430-0050 (54-71-01) for 600 cm^2, contains:
1 x 10 mL KPL LumiGLO Reserve Substrate Solution A
1 x 20 mL KPL LumiGLO Reserve Substrate Solution B
1 x 100 mL KPL Wash Solution Concentrate (20X)

5430-0051 (54-71-02) for 1000 cm^2, contains:
1 x 17 mL KPL LumiGLO Reserve Substrate Solution A
1 x 34 mL KPL LumiGLO Reserve Substrate Solution B
1 x 100 mL KPL Wash Solution Concentrate (20X)

STORAGE/STABILITY

KPL LumiGLO Reserve Chemiluminescent Substrate is supplied as a two component substrate system and concentrated wash buffer. Store all components at 2-8°C. KPL LumiGLO Reserve Solution A should remain stored in its original container and protected from light. Minimize contact with metallic surfaces. Stable for a minimum of one year from date of receipt when stored under proper conditions. Prepared KPL LumiGLO Reserve working solution is stable for several hours at room temperature when protected from light.

PRODUCT PREPARATION

KPL LumiGLO Reserve Working Solution:

- Mix 1 part Solution A (luminol solution) to 2 parts Solution B (reaction buffer) v/v.
- Mix well and protect working solution from intense light.
- For best results, allow the KPL LumiGLO Reserve working solution to warm to room temperature prior to use.

1X KPL Wash Solution:

- Dilute 20X KPL Wash Solution Concentrate 1/20 with reagent quality water.

KPL LUMIGLO RESERVE CHEMILUMINESCENT SUBSTRATE USER'S GUIDE

- KPL LumiGLO Reserve can be used with nitrocellulose and PVDF membranes. For maximum signal to noise, nitrocellulose is recommended.
- The KPL LumiGLO Reserve working solution should be protected from light after preparation and warmed to room temperature prior to its use.
- For maximum signal, expose membrane to film immediately after incubation with KPL LumiGLO Reserve. The reaction and film exposure are performed at room temperature. For most applications, exposures of 10 minutes or less produce sufficient sensitivity.
- KPL LumiGLO Reserve is an extremely sensitive substrate. Insufficient washing of membranes or contamination of substrate with HRP will result in non-specific background.
- Because of KPL P qmKPS Vi ivi tiv

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sensitivity, it is imperative to the success of the assay that the HRP conjugate be titrated to give the optimal signal to noise.

- Do not allow KPL LumiGLO Reserve to contact the film. If this occurs, LumiGLO Reserve solution will cause dark spots to appear on the film.

KPL LumiGLO Reserve emits light over the course of 4 8 hours with the most intense emission within the first hour. Because of its high light intensity, most images may be captured well within 10 minutes making multiple exposures easy to obtain.

APPLICATIONS

KPL LumiGLO Reserve Chemiluminescent Substrate has been optimized for Western blotting and dot blotting applications. It is also suitable for use in microwell applications such as ELISA. The following is a recommended procedure for Western blot detection.

WESTERN BLOT DETECTION

There are many protocols available for the detection of Western blots. Many blocks and wash solutions have been successfully used with KPL LumiGLO Reserve, including KPL SignaLOCK™ Blocking Solution, KPL Detector™ Block, milk and BSA blocks and TBST washes and diluents, respectively. For optimal signal to noise and sensitivity, the KPL Wash Solution Concentrate contained in this kit should be used. The following protocol and reagents are recommended.

Suggested Reagents/Equipment Not Included

1. Primary antibody
2. HRP-labeled secondary antibody
3. Nitrocellulose or PVDF membrane
4. Blocking Solution (See RELATED PRODUCTS)
5. X-ray film (double emulsion) or CCD Imager
6. Platform shaker or rocker
7. Developing chemicals/equipment
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 1/10,000 to 1/100,000 of a 0.1 mg/mL stock.

WESTERN BLOT DETECTIONAT A GLANCE

Total time: 4 hours

Polyacrylamide Gel Electrophoresis

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Immobilize Protein on Membrane

↓

Block Membrane
1 hour or overnight

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Incubate Primary Antibody
1 hour

↓

Wash Membrane
3 x 5 minutes
1 x 10 minutes

↓

Incubate Conjugate
1 hour

↓

Wash Membrane
3 x 5 minutes
1 x 10 minutes

↓

Incubate LumiGLO Reserve Substrate
1 minute

↓

Expose to Film
10 seconds - 10 minutes

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STEPS	CRITICAL POINTS
1. Block the membrane by immersing in block solution (1X KPL Detector Block is recommended) using a minimum of 0.2 mL/cm^2 of membrane. Block at room temperature for 1 hour with gentle rocking or shaking, or stationary at 2-8°C overnight.	*Example: for a 10 x 10 cm blot, use 20 mL of block. Make sure to use a container of proper size that allows the block solution to freely float over the membrane.*
2. Incubate membrane with primary antibody or serum sample for at least 1 hour. This antibody should be added directly to the Block Solution that was used for blocking (Step 1).	*It is recommended that serial dilutions through a dot blot be performed to determine the optimal working dilution, or use the concentration determined by the primary antibody supplier.*
3. Wash the membrane in a generous amount of 1X KPL Wash Solution (at least 25mL for a 100 cm^2 membrane). Wash membrane 3 times for 5 minutes each, followed by one 10-minute wash.	*This solution will provide optimal signal to noise. 1X TBS/PBS-TWEEN may also be used.*
4. Dilute appropriate conjugate 1/10,000 1/100,000 (of a 0.1 mg/mL stock) in freshly prepared conjugate diluent using a minimum of 0.2 mL/cm^2 of membrane.	*Example: 2 µL conjugate + 20 mL diluent. Suggested diluents include KPL Detector Block and TBS/PBSTWEEN. The optimal dilution may vary for different lots of conjugate. It is imperative that you titrate the conjugate to determine the optimal working dilution.*

STEPS	CRITICAL POINTS
5. Incubate blot with diluted conjugate for one hour at room temperature.	
6. During the conjugate incubation step, prepare KPL LumiGLO Reserve. Add 1 part of solution A with 2 parts of solution B. Prepare 0.05 mL/cm^2 membrane to be detected.	*Prepare KPL LumiGLO Reserve in advance to allow it to come to room temperature prior to its use. Cover it with foil to minimize exposure to light.*
7. After the conjugate incubation, wash as described in step 3.	
8. Pour off the remaining wash buffer from the blot and place the membrane on a sheet protector or a dry tray.	
9. Gently pipette 0.05 mL/cm^2 of previously prepared KPL LumiGLO Reserve over the entire membrane. Incubate ***without*** rocking for 1 minute.	*Example: for a 10 x 10 cm blot, use 5 mL of KPL LumiGLO Reserve. The surface tension of the substrate will keep it on the surface of the membrane.*
10. Lift the membrane with forceps and blot the excess substrate onto a piece of filter paper. Seal the membrane in clear plastic and expose to X-ray film for 10 seconds to 1 minute. Adjust exposure time as needed.	*Excessive substrate on the blot will contribute to background.* *Take caution to ensure the surface of the membrane to which the assay reagents were applied is facing the film. Do not allow the film to get wet, nor move during exposure.*

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STEPS	CRITICAL POINTS
	Optimal exposure time should be determined by the signal to noise ratio and the amount of conjugate used. When using greater amounts of conjugate, 10 seconds may provide acceptable results.
11. ***Optional:*** Chemiluminescent Imager Detection. Incubate the blot for twice the time typically used for film. If the imager provides stacking capabilities, capture exposures at 5 minute intervals for 1 hour to maximize signal. The optimal exposure can be chosen.	*Follow the manufacturer's recommendations regarding the set up and operation of the imager.*

TROUBLESHOOTING

Problem 1: No Signal

Possible Cause	Corrective Measure
• Inactive horseradish peroxidase	Verify enzyme activity by mixing 10 P sj hmp i h 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 on unblocked membrane to verify attachment of target protein or use a pre stained protein marker to monitor transfer.
• Detection of non-blotted side of membrane	Ensure correct orientation of the membrane during the assay and film exposure.
• 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.

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Possible Cause	Corrective Measure
• Insufficient incubation of primary antibody to target	Increase the incubation times for weak primary antibodies.
• Insufficient exposure time	Increase the time of exposure to film.
• Excessive washing beyond recommended procedure	Follow the procedure as written.

Problem 3: Excessive signal, nonspecific bands or general background

Possible Cause	Corrective Measure
• Overexposure of film to signal	Expose the membrane to film 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 1/10,000 1/100,000 of a 0.1 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.

Possible Cause	Corrective Measure
• Endogenous peroxidase in the sample	Test by incubating the blocked membrane in KPL LumiGLO Reserve (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	Jsps qer jeg viv recommended procedure or contact the manufacturer for additional support regarding the blotting apparatus.
• Excessive substrate	Remove excess substrate before exposure of the membrane to film.
• Ghost images from shifted position of film during development	Avoid movement of film over membrane during exposure period.
• 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.*[11]. After performing protein transfer, detection with KPL LumiGLO Reserve and film exposure, membranes may be stripped and reprobed with new primary and secondary antibodies.