KPL PhosphaGLO Reserve AP Substrate

Catalog No.	Size
5430-0052 (55-60-01)	30 mL
5430-0053 (55-60-02)	100 mL

DESCRIPTION

KPL PhosphaGLO AP Substrate contains a dioxetane-based chemiluminescent substrate designed for use with phosphatase-labeled (AP) reporter molecules. KPL PhosphaGLO Reserve offers great improvements in the way of signal intensity. These products are specifically designed for the detection of proteins that are (1) difficult to detect because they are in such low quantities or (2) are from samples that are precious.

KPL PhosphaGLO Reserve AP Substrate is provided as a stable one-component solution. The product 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 on Western blots and ELISAs.

Two sizes are available. Results can be obtained on X-ray film or a chemiluminescent imager to provide a permanent record. A luminometer should be used for ELISA.

NOTE: This product may arrive frozen.

CONTENTS

5430-0052 (55-60-01) contains:
1 x 30 mL KPL PhosphaGLO Reserve AP Substrate

5430-0053 (55-60-02) contains:
1 x 100 mL KPL PhosphaGLO Reserve AP Substrate

STORAGE/STABILITY

Store this product at 2 8°C. If it arrives frozen, allow it to thaw prior to use. KPL PhosphaGLO Reserve Solution should remain stored in its original container and protected from light.

The product is stable for a minimum of two years from date of receipt when stored under proper conditions.

KPL PHOSPHAGLO RESERVE CHEMILUMINESCENT

KPL PhosphaGLO Reserve can be used with nitrocellulose and PVDF membranes. For best results, nitrocellulose is recommended.

The KPL PhosphaGLO Reserve working solution should be protected from light and warmed to room temperature prior to use.

KPL PhosphaGLO Reserve is an extremely sensitive substrate. Insufficient washing of membranes or contamination of substrate with AP will result in non-specific background.

Because of KPL PhosphaGLO Reserve super sensitivity, the AP conjugate should be titrated to give the best results.

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

KPL PhosphaGLO Reserve emits light over the course of 5-7 days. Because of its high light intensity, images may be captured over the course of many different intervals. Initially, perform a 1 minute and a 10 minute exposure.

APPLICATIONS

KPL PhosphaGLO Reserve 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

Suggested Reagents/Equipment Not Included

1. Primary antibody
2. AP-labeled secondary
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
9. Wash solution (see RELATED PRODUCTS)
10. Assay buffer (see RELATED PRODUCTS)

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CONJUGATE OPTIMIZATION PRIOR TO DETECTION

Before beginning the assay, the optimal conjugate dilution should be determined. 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 should be optimized, as slight differences in activity can produce 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 DETECTION AT A GLANCE

Total time: 4 hours

Polyacrylamide Gel Electrophoresis

Immobilize Protein on Membrane

Block Membrane
1 hour or overnight

Incubate with Primary Antibody
1 hour

Wash Membrane
3 x 5 minutes
1 x 10 minutes

Incubate with Conjugate
30 minutes - 1 hour

Wash Membrane
3 x 5 minutes
1 x 10 minutes

Rinse with Assay buffer
2 x 2 minutes

Incubate with KPL PhosphaGLO Reserve Substrate
1 minute

Expose to Film
10 seconds - 10 minutes

PACKAGE INSERT

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TROUBLESHOOTING

Problem 1: No Signal

Possible Cause	Corrective Measure

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Stripping and Re-probing a Western Blot
This protocol is adapted from Kaufmann, *et. al*.[11]. After performing protein transfer, detection with KPL PhosphaGLO Reserve and film exposure, membranes may be stripped and re-probed 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 -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 Block Solution.
4. Repeat detection procedure.

PRODUCT SAFETY AND HANDLING
See SDS (Safety Data Sheet) for this product.

RELATED PRODUCTS	CAT. NO.
KPL PhosphaGLO AP Substrate	5430-0054 (55-60-03)
KPL Detector Block	5920-0004 (71-83-00)
KPL Milk Diluent/Blocking Solution	5140-0011 (50-82-01)
KPL 10% BSA Diluent/Blocking Solution	5140-0006 (50-61-00)
KPL Wash Solution	5150-0008 (50-63-00)
KPL Coating Solution	5150-0014 (50-84-00)
KPL Phosphatase Assay Buffer	5960-0017 (50-63-14)

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

REFERENCES

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