KPL LumiGLO®
Chemiluminescent Substrate

Catalog No.	Size
5430-0042 (54-61-02)	60 mL
5430-0040 (54-61-00)	240 mL
5430-0041 (54-61-01)	720 mL

DESCRIPTION

KPL LumiGLO® is a luminol-based chemiluminescent substrate designed for use with peroxidase-labeled (HRP) reporter molecules. KPL LumiGLO provides increased sensitivity over chromogenic substrates in both blotting and microwell assays. Positive reaction sites are rapidly detected with high sensitivity and minimal background. In blotting applications, permanent results are recorded on X-ray film. The use of KPL LumiGLO allows for multiple stripping and reprobing of blots. In microwell assays, positive reactions are rapidly detected and read in a Luminometer. KPL LumiGLO provides a dynamic range that is linear for a longer period of time than other chemiluminescent substrates.

In the presence of hydrogen peroxide, HRP converts luminol to an excited intermediate dianion. This dianion emits light on return to its ground state. After reaction with HRP the light emission from KPL LumiGLO reaches maximum intensity within 5 minutes and is sustained for approximately 1 – 2 hours.

CONTENT

- 5430-0042 (54-61-02) contains:
  - 1 x 30 mL KPL LumiGLO Substrate A
  - 1 x 30 mL KPL LumiGLO Substrate B

Sufficient material is supplied to process approximately 600cm^2 of membrane.

- 5430-0040 (54-61-00) contains:
  - 1 x 120 mL KPL LumiGLO Substrate A
  - 1 x 120 mL KPL LumiGLO Substrate B

Sufficient material is supplied to process approximately 2400cm^2 of membrane.

- 5430-0041 (54-61-01) contains:
  - 3 x 120 mL KPL LumiGLO Substrate A
  - 3 x 120 mL KPL LumiGLO Substrate B

Sufficient material is supplied to process approximately 7200cm^2 of membrane.

STORAGE/STABILITY

KPL LumiGLO is supplied as a two component system. Store at 2-8 °C. Stable for a minimum of one year from date of receipt when stored at 2-8 °C.

APPLICATIONS

KPL LumiGLO can be used in both microwell and blotting applications such as ELISA, Western blotting, Southern blotting, dot blotting, plaque and colony hybridizations.

SUGGESTED REAGENTS NOT INCLUDED

1. Primary antibody or DNA probe.
2. HRP-labeled antibody or streptavidin.
3. X-ray film.
4. Nylon, nitrocellulose or PVDF membrane.
5. Blocking Solution (See RELATED PRODUCTS).
6. Wash Solution (See RELATED PRODUCTS).
7. 20X SSC (See RELATED PRODUCTS).
8. KPL Protein Detector™ LumiGLO Western Blotting Kit provides HRP secondary antibodies, blocking solution, wash solution and KPL LumiGLO Chemiluminescent Substrate (See RELATED PRODUCTS).
9. KPL Detector™ HRP Chemiluminescent Blotting Kit (See RELATED PRODUCTS).

PREPARATION

- Mix Substrate A and Substrate B in equal volumes.
- Warm to room temperature before use.
- Solution need not be protected from light.
- Solution is stable for up to one hour at room temperature or up to 24 hours when stored at 2-8 °C.

BLOTTING PROCEDURES

All steps are at room temperature unless otherwise noted.

Note: KPL LumiGLO can be used with nitrocellulose, nylon and PVDF membranes.

Note: Milk or casein-based blocking solutions are recommended for use with KPL LumiGLO. BSA or serum-based blocking agents may cause elevated background. SeraCare recommends KPL Detector Block (See RELATED PRODUCTS) for the highest sensitivity with low background.

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WESTERN BLOTTING

Detection:

1. Perform gel electrophoresis and transfer following standard procedures.
2. Block the membrane with KPL Detector Block (See RELATED PRODUCTS) or other appropriate blocking solution, for 1 hour at room temperature or overnight at 2- 8°C.
3. Incubate membrane with primary antibody or serum sample, diluted in blocking solution, for 1 hour.
4. Wash membrane with KPL Wash Solution (See RELATED PRODUCTS) or other appropriate wash solution, 3 times for 5 minutes each.
5. Incubate with HRP conjugate, diluted in blocking solution, for 1 hour. The concentration of HRP conjugate must be determined experimentally.
6. Wash 3 times for 5 minutes each.
7. Prepare KPL LumiGLO Chemiluminescent Substrate by mixing equal volumes of Substrates A and B. Incubate membrane for 1 minute in the KPL LumiGLO working solution (use approximately 1 mL per 10 cm^2 membrane).
8. Remove membrane from KPL LumiGLO and touch the corner to a piece of filter paper. Place membrane between plastic sheets or in a hybridization bag.
9. Expose membrane to X-ray film 1 - 10 minutes. The signal obtained from the first exposure will allow the researcher to determine an exposure time for optimal signal.

Stripping and Reprobing a Western blot:

1. Remove membrane from plastic following initial detection with KPL LumiGLO.
2. Rinse membrane for 30 - 90 minutes at 70°C in 2% SDS (w/v)/62.5 mM Tris-HCl (pH 6.8 at 20°C)/100 mM β-mercaptoethanol.
3. Wash membrane 2 times in 10 mM Tris-HCl (pH 7.4 at 20°C)/150 mM NaCl.
4. Block membrane for 2.5 hours with KPL Detector Block or 10 mM Tris-HCl (pH 7.4 at 20°C)/150 mM NaCl/5% nonfat dry milk.
5. Repeat detection procedure.

SOUTHERN BLOTTING

Detection:

1. Perform gel electrophoresis and transfer following standard procedures.
2. Prehybridize membrane for 30 minutes to 1 hour at the appropriate hybridization temperature.
3. Add biotinylated probe to hybridization solution and hybridize 3 - 16 hours at the appropriate hybridization temperature.
4. Following hybridization perform stringency washes with SSC or SSPE following standard protocols.
5. Block membrane for 30 minutes to 1 hour with KPL Detector Block (See RELATED PRODUCTS) or other appropriate blocking solution.
6. Incubate with KPL HRP-Streptavidin (See RELATED PRODUCTS), diluted in blocking solution, for 20 – 30 minutes. The optimal concentration of KPL HRP-Streptavidin must be determined experimentally.
7. Transfer membrane to a clean container and wash with KPL Biotin Wash Solution (See RELATED PRODUCTS) or other appropriate wash solution. Perform 3 washes for 5 minutes each.
8. Prepare KPL LumiGLO Chemiluminescent Substrate by mixing equal volumes of Substrates A and B. Incubate membrane for 1 minute in the KPL LumiGLO working solution (use approximately 1 mL per 10 cm^2 membrane).
9. Remove membrane from KPL LumiGLO and touch the corner to a piece of filter paper. Place membrane between plastic sheets or in a hybridization bag.
10. Expose membrane to X-ray film. KPL recommends an initial exposure of 10 - 15 minutes for plasmid DNA and 30 - 60 minutes for genomic DNA. The signal obtained from the first exposure will allow the researcher to determine an exposure time for optimal signal.

Stripping and Reprobing a Southern Blot:

1. Remove membrane from plastic sheets following initial detection with KPL LumiGLO.
2. Rinse membrane for 5 minutes in wash solution.
3. Incubate membrane in 0.2N NaOH/0.1% SDS (pre-warmed to 55°C) for 20 minutes at 55°C.
4. Rinse membrane twice for 5 minutes each in 2X SSC. Stripped membranes can be stored dry at room temperature or refrigerated.
5. Repeat detection procedure.

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TROUBLESHOOTING: BLOTTING

Problem	Corrective Measure
Excess Signal or Background	• Decrease film exposure time. • Decrease HRP conjugate concentration. • Reduce conjugate incubation time. • Increase washing or blocking times. • Load less protein/DNA onto gel.
No signal	• Verify transfer by staining protein gel with Coomassie blue or DNA gel with ethidium bromide. • Verify protein transfer by staining membrane with Ponceau-S or Amido black. • Make sure HRP secondary antibody is specific for the primary antibody. • Do not add sodium azide to solutions, this will inhibit peroxidase activity.
Weak signal	• Increase film exposure time. • Increase conjugate concentration. • Increase conjugate incubation time. • Load more protein/DNA onto gel. • Make sure primary antibody has high affinity for target protein. Antibody affinity may change after denaturation of sample with SDS.

ELISA PROCEDURE

All steps are at room temperature unless otherwise noted.

Note: The typical light decay of KPL LumiGLO in microtiter plates has a $t_{1/2}$ value of 60 minutes.

1. Coat an opaque white microwell plate with 100 µL/well antigen, diluted in KPL Coating Solution (See RELATED PRODUCTS) or Carbonate Buffer (pH 9.6), for 2 hours at room temperature or overnight at 2-8°C. Optimal antigen dilution must be determined experimentally.
2. Block plate for 15 - 30 minutes using 300 µL/well of KPL Milk Diluent/Blocking Solution (See RELATED PRODUCTS) or other appropriate blocking solution.
3. Incubate plate for 1 hour with 100 µL/well primary antibody diluted in blocking solution. Optimal antibody dilution must be determined experimentally.
4. Wash plate 3 times with KPL Wash Solution **(**See RELATED PRODUCTS) or other appropriate wash solution.
5. Incubate plate for 30 minutes to 1 hour with 100 µL/well HRP-labeled secondary antibody diluted in blocking solution or other appropriate diluent. Optimal antibody dilution must be determined experimentally.
6. Wash plate 3 times.
7. Prepare KPL LumiGLO Chemiluminescent Substrate by mixing equal volumes of Substrate A and Substrate B. Add 100 µL/well KPL LumiGLO working solution.
8. Read on a Luminometer with 1 second integration time per well. KPL LumiGLO provides consistent results when read 5 - 45 minutes after addition of substrate.

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TROUBLESHOOTING: ELISA

Problem	Corrective Measure
Excess Signal or Background	• Decrease HRP conjugate concentration. • Reduce conjugate incubation times. • Increase washing or blocking times. • Decrease the amount of protein coated to plate. • High signal wells can contaminate adjacent wells. Remove substrate from suspect well and place in another well to get a more accurate reading.
No signal	• Make sure HRP secondary antibody is specific for the primary antibody. • Do not add sodium azide to solutions, this will inhibit peroxidase activity. • Verify the Luminometer is working correctly
Weak signal	• Increase conjugate concentration. • Increase conjugate incubation time. • Increase the amount of protein coated to the plate. • Make sure primary antibody has high affinity for target protein.

PRODUCT SAFETY AND HANDLING

See SDS (Safety Data Sheet) for this product.

REFERENCES

1. Towbin, H. et. al. (1979) Electrophoretic Transfer of Proteins From Polyacrylamide Gels to Nitrocellulose Sheets. Proc. Natl. Acad. Sci. 76: 4350-4354.
2. Reinhart, M. and Malamud, D. (1982) Protein Transfer From Isoelectric Focusing Gels: The Native Blot. Anal. Biochem. 123: 229-235.
3. Kricka, L. (1991) Chemiluminescent and Bioluminescent Techniques. Clin. Chem. 37(9): 1472-1481.
4. Knecht, D. and Dimond, R. (1984) Visualization of Antigenic Proteins on Western Blots. Anal. Biochem. 136: 180-184.
5. Burnette, W. (1981) Western Blotting: Electrophoretic Transfer of Proteins and Nucleic Acids From Slab Gels to Unmodified Nitrocellulose and Radiographic Detection With Antibody and Radioiodinated Protein A. Anal. Biochem. 102: 459-471.
6. Kaufmann, et. al. (1987). The Erasable Western Blot. Anal. Biochem. 161: 89-95.
7. Isacsson V, Wettermark, G. (1974) Chemiluminescence in Analytical Chemistry. Anal. Chim. Acta. 68: 339-362.
8. Ausubel, R., et. al. (eds.) Current Protocols in Molecular Biology. John Wiley and Sons, NY.

RELATED PRODUCTS	CAT NO.
KPL Detector Block	5920-0004 (71-83-00)
KPL Wash Solution Concentrate	5150-0008 (50-63-00)
KPL HRP Streptavidin, MB grade	5950-0004 (474-3000)
KPL Biotin Wash Solution Concentrate	5960-0015 (50-63-06)
KPL 20X SSC	5960-0021 (50-86-05)
KPL Coating Solution Concentrate	5150-0014 (50-84-00)
KPL Milk Diluent/Blocking Solution	5140-0011 (50-82-01)
KPL Biodyne B Nylon Membrane	5960-0026 (60-00-51)
KPL Protein Detector LumiGLO Western Blotting Kit	5410-0009 (54-12-50)
KPL Detector™ HRP Chemiluminescent Blotting Kit	5910-0027 (54-30-00)