

Certificate of Analysis

LumiGLO[®] Chemiluminescent Substrate Catalog # 20-212

Product Description: Chemiluminescent substrate designed for use with peroxidase labeled (HRP) reporter molecules.

Contents: 50ml Chemiluminescent Substrate A (Catalog # 20-212a), 50ml Chemiluminescent Substrate B (Catalog # 20-212b). Sufficient material to process approximately 1,000cm² of membrane.

Storage and Stability: LumiGLO[®] is supplied as a two-component system. Store at 4°C. Stable for a minimum of one year from date of receipt when stored at 4°C.

Note: LumiGLO[®] Chemiluminescent Substrate A is a liquid irritant. Read the MSDS before use.

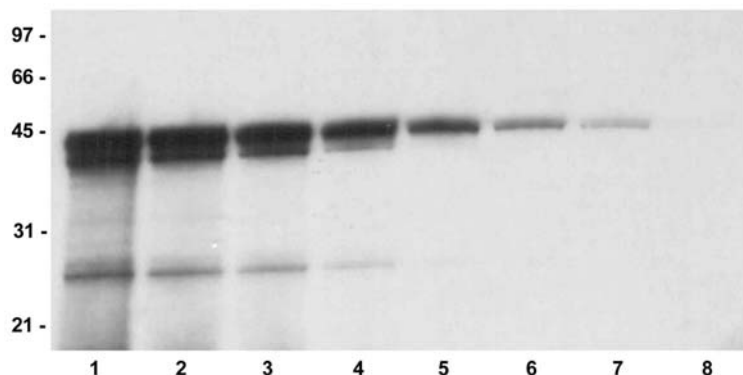
**FOR RESEARCH USE ONLY
NOT FOR USE IN HUMANS**

Quality Control Testing

Immunoblot Analysis: 20, 10, 5, 2.5, 1.0, 0.5, 0.25, and 0.1µg of rat L6 cell lysates were probed with 0.5µg/ml of anti-MAP Kinase 1/2 (Catalog # 06-182). Map Kinase 1/2 was detected in 0.25-20µg of cell lysate using LumiGLO[®] Chemiluminescent Substrate for visualization.

Additional Research Applications

LumiGLO[®] has been reported to be used in both microwell and blotting applications such as ELISA, Southern blotting, dot blotting, plaque and colony hybridizations.



Immunoblot Analysis

L6 cell lysates, 20µg (lane 1), 10µg (lane 2), 5µg (lane 3), 2.5µg (lane 4), 1.0µg (lane 5), 0.5µg (lane 6), 0.25µg (lane 7), and 0.1µg (lane 8) were resolved by electrophoresis, transferred to nitrocellulose and probed with Anti-MAP Kinase 1/2 (0.5µg/ml). Proteins were visualized using a goat anti-rabbit secondary antibody conjugated to HRP. LumiGLO[®] chemiluminescent substrate was used as the detection system. Arrow indicates MAP Kinase 1/2 (42-44kDa).

General Reference:

Towbin, H., *et al.*, Proc. Natl. Acad. Sci. **76:** 4350-4354, 1979.

LumiGLO[®] is a registered trademark of Kirkegaard & Perry Laboratories, Inc.

Preparation of LumiGLO®

- Mix Substrate A and Substrate B in equal volumes (use approximately 1 ml per 10 cm² membrane).
- Warm to room temperature before use.
- Solution need not be protected from light.
- Solution is stable for up to one hour when stored at room temperature, and up to 24 hours when stored at 4°C

Immunoblot Protocol

1. Perform SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on a cell lysate sample (cell lysis buffer: 50mM Tris-HCl, pH 7.4; 1% NP-40; 0.25% sodium deoxycholate; 150mM NaCl; 1mM EDTA; 1mM PMSF; 1µg/ml each aprotinin, leupeptin, pepstatin; 1mM Na₃VO₄; 1mM NaF) and transfer the proteins to nitrocellulose. Wash the blotted nitrocellulose twice with water.
2. Block the blotted nitrocellulose in freshly prepared PBS containing 3% nonfat dry milk (Catalog # 20-200), (PBS-MLK) for 30 minutes at room temperature with constant agitation.
3. Incubate the nitrocellulose with primary antibody of choice, diluted in freshly prepared PBS-MLK overnight with agitation at 4°C.
4. Wash the nitrocellulose twice with water.
5. Incubate the nitrocellulose in the HRP conjugate secondary reagent of choice in PBS-MLK for 1.5 hours at room temperature with agitation. The concentration of HRP conjugate must be determined experimentally.
6. Wash the nitrocellulose twice with water.
7. Wash the nitrocellulose in PBS-0.05% Tween 20 for 3-5 minutes.
8. Rinse the nitrocellulose in 4-5 changes of water.
9. Prepare LumiGLO® chemiluminescent Substrate by mixing equal volumes of Substrates A and B (See notes above). Incubate membrane for 1 minute in the LumiGLO® working solution (use approximately 1 ml per 10 cm² membrane).
10. Remove membrane from LumiGLO® and touch the corner to a piece of filter paper. Place membrane on opened Film Exposure Folder (Catalog # 20-211). Close folder, making sure there are no air bubbles.
11. Expose membrane to X-ray film. The signal obtained from the first exposure will allow the researcher to determine an exposure time for optimal signal.