

KemiLumin Sirius

SUBSTRATES

Chemiluminescent HRP Substrate

Cat. No. 5410

Product Characteristics

Luminol (5-amino-2,3-dihydrophthalazine-1,4-dione) is a chemiluminescence substrate for Horseradish Peroxidase (HRP). Luminol-based chemiluminescence is a chemical reaction that produces visible blue light at $\lambda = 425 \text{ nm}$, when catalyzed by the presence of HRP. Quantification of the reaction both in immunoassays and Western blotting occurs by measurement of relative light units (RLU) using a chemiluminescence reader.

KemiLumin Sirius is exceptional for higher assay sensitivity, which produces an intensive stable light, while offering a highly stable working solution, low signal to noise ratio, and low background characteristics. The concentration of all chemicals in KemiLumin Sirius is below hazardous levels making it compliant with REACH Restricted Substances List Annex XVII, while ensuring maximal safety during use, and minimal negative environmental impact.

Composition & Properties

KemiLumin Sirius consists of two components, A and B.

Component A: The component contains luminol and appropriate enhancers.

Component B: The component contains peroxide. The two components must be mixed 1:1 for preparation of the working solution. No other reagents should be added.

Working Procedure

The desired amount of working solution is made by mixing component A and B 1:1 before it is poured into a sealed container and allowed to reach room temperature in the dark before use.

Immunoassays: The recommended volume of working solution per microtiter well is 50 μL to 200 μL . The volume

is added to the well and the reaction is instantaneous. Recommended incubation times are between 5 and 25 min. The plate should be kept in the dark during incubation.

Endpoint Assays: Measurements are made after the chosen incubation time. It is recommended not to exceed 30 min between addition of the substrate and measurement.

Kinetic Immunoassays: Measurements can start directly after addition of substrate.

Western Blot: The membrane is covered with the working solution (approximately 100 $\mu\text{L}/\text{cm}^2$). The recommended incubation time is 2 min (20/min on a horizontal shaker) after which the working solution is decanted and the membrane wrapped into a foil. Pictures can be taken immediately with an exposure time of approximately 2 sec, but up to 60 min can be used if needed.

Tips & Tricks

- If reduced intensity is required, it is recommended to shorten the time of development or to choose KemiLumin Vega (Cat. No. 5420).
- To eliminate cross-talk and to obtain low background signals it is recommended to use black ELISA plates for immunoassays.

Handling & Storage

- The working solution is stable for 48 hours in the dark at room temperature and for 72 hours when stored in the dark at 2-8 °C.
- Avoid exposure to light, heat, and contamination with peroxidase.
- Re-dispense only into bottles made of High-Density Polyethylene (HDPE), amber color for component A and the working solution, while natural color can be used for component B.



Our Immunoassay Solutions are eco-friendly, creating a healthy work environment and preserving natural resources, while helping our customers fulfill significant regulatory requirements. **We call it ECO-TEK.**