20X LumiGLO® Reagent and 20X Peroxide



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For Research Use Only. Not For Use In Diagnostic Procedures.

Product Includes	Product #	Quantity
7003P		
LumiGIO® Reagent A (20X)	95538P	5 ml
Peroxide Reagent B (20X)	39864P	5 ml
7003\$		
LumiGIO® Reagent A (20X)	95538S	25 ml
Peroxide Reagent B (20X)	39864S	25 ml

Background: Chemiluminescent detection systems have emerged as the best all-around method for detection of Western blots. They eliminate the hazards associated with radioactive materials and toxic chromogenic substrates. The speed and sensitivity of these methods are unequalled by traditional alternatives. Because results are generated on film, it is possible to record and store data permanently, and blots detected with chemiluminescent methods are easily stripped for subsequent reprobing with additional antibodies. Horseradish peroxidase (HRP) conjugated secondary antibodies are utilized in conjunction with specific chemiluminescent substrates to generate the light signal. Horseradish peroxidase-antibody conjugates have a very high turnover rate, giving good sensitivity with short reaction times.

Description: LumiGLO® chemiluminescent substrate is a luminol-based system designed for use with our Phototope®-HRP detection assays utilizing peroxidase-labeled antibodies immobilized on membranes. In the presence of hydrogen peroxide, horseradish peroxidase (HRP) converts luminol to an excited intermediate dianion. This dianion emits light on return to its ground state. Light emission is maximal immediately after exposure of the substrate to HRP and continues for 0.5-1 hour. Light can be captured on X-ray film, typically by exposure for a few seconds. Maximum sensitivity can be obtained by longer exposure.

*Avoid repeated exposure to skin (see enclosed Material Safety Data Sheet or refer to our website for further information).

Storage: Store at 4°C.

Direction for Use:

- (a) Wash membrane-bound HRP (antibody conjugate) three times, for 5 minutes in TBS/T.
- (b) Prepare substrate by diluting LumiGIO® Reagent A (20X) (#95538) and Peroxide Reagent (20X) (#39864) to 1X in water (e.g. For 10 ml, add 0.5 ml LumiGLO® and 0.5 ml peroxide to 9.0 ml water).
- (c) Incubate substrate with membrane for 1 minute, remove excess solution (membrane remains wet), wrap in plastic and expose to X-ray film.

Solutions and Reagents:

Note: Prepare solutions with Milli-Q® or equivalently purified water.

Chemiluminescent
Substrate

2° Ab

1° Ab

Protein

Membrane

After the primary antibody is bound to the target protein, a complex with HRP-linked secondary antibody is formed. The LumiGLO® is added and emits light during enzyme catalyzed decomposition.

Phototope® is a trademark of Cell Signaling Technology, Inc. LumiGLO™ is a trademark of Kirkegaard & Perry Laboratories (KPL). Milli-Q™ is a trademark of Millipore.