

**#7003**  
 Store at +4C

## 20X LumiGLO<sup>®</sup> Reagent and 20X Peroxide



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Petite 10 ml (5 ml each substrate)  
Small 50 ml (25 ml each substrate)

**For Research Use Only. Not for Use in Diagnostic Procedures.**

Product Includes	Product #	Quantity (Small)	Quantity (Petite)
LumiGLO(R) Reagent A (20X)	95538	1 x 25 ml	1 x 5 ml
Peroxide Reagent B (20X)	39864	1 x 25 ml	1 x 5 ml

### Description

LumiGLO<sup>®\*</sup> chemiluminescent substrate is a luminol-based system designed for use with our Phototope<sup>®</sup>-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 up to 3 hours. 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. This product is stable for 12 months. DO NOT store Reagents A and B pre-mixed. Reagents A and B should be combined just prior to exposing membranes.

### Background

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

### Directions for Use

- Wash membrane-bound HRP (antibody conjugate) three times, for 5 minutes in TBS/T.
- Prepare substrate by diluting 20X LumiGLO<sup>®</sup> and 20X Peroxide to 1X in water (e.g. for 10 ml, add 0.5 ml LumiGLO<sup>®</sup> and 0.5 ml peroxide to 9.0 ml water).
- 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

Prepare solutions with Milli-Q<sup>®</sup> or equivalently purified water. Wash Buffer (TBS/T): 20 mM Tris-HCl (pH 7.6), 137 mM NaCl and 0.1% Tween-20. Advantages of the Phototope<sup>®</sup> Western Detection System- Sensitivity: Detection of sub-picogram amounts of protein is routine with good primary antisera.- Speed: Less than 1 hour is required for the entire detection procedure. Exposure times are seconds to minutes.- Multiple Exposures: Light is emitted at a constant rate for several minutes, so you can perform multiple exposures to optimize signal intensity. Re-exposure at a future date is achieved by simply adding more reagent.

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