

# PhosphaGLO™

## See More of What You've Been Missing!

For Western blotting results that jump right out at you, take a closer look at the advantages of PhosphaGLO™ AP Substrates.

To meet your needs for detection of proteins using Western blotting, KPL offers two chemiluminescent substrates designed for use with alkaline phosphatase conjugates. Both provide great sensitivity and unmatched convenience whether you are designing a new protocol or optimizing an existing one.

### Great Sensitivity

With sensitivity in the femtogram range, PhosphaGLO Reserve™ AP Substrate is ideal for those situations where the protein of interest is expressed in low concentrations. PhosphaGLO AP Substrate is the substrate of choice for routine detection of proteins in the picogram range.

### Low Background Without Special Blockers

Whether you use nitrocellulose or PVDF membranes, PhosphaGLO AP Substrates provide superior signal with low background. No special blockers are needed.

### Convenience

Both PhosphaGLO AP Substrates offer exceptional convenience:

- Ready to use! Substrates are formulated as one-component solutions; no need to mix or dilute.
- Substrates are stable at 4° C for two years.
- Results can be recorded on film or with a chemiluminescent imager.

### Simplified Assay Development

Assay development with PhosphaGLO AP Substrates is facilitated by their long glow times, up to five days. Repeated exposures are possible. The substrates are provided with an easy-to-follow protocol. PhosphaGLO AP Substrates can, in most cases, be substituted for other AP chemiluminescent substrates with minimal optimization.

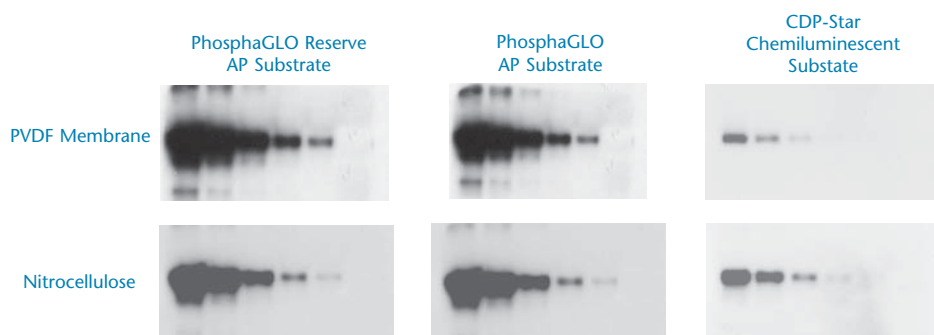


## PhosphaGLO AP Substrates - Product Information

	PhosphaGLO Reserve	PhosphaGLO
Detection Limit	Femtogram	Picogram
Emission Duration	> 5 days	> 5 days
Stability	2 years at 4°C	2 years at 4°C
Recommended Membranes	Nitrocellulose and PVDF	Nitrocellulose and PVDF
Detection Methods	Film/Chemiluminescent Imager	Film/Chemiluminescent Imager

### Comparison of AP Chemiluminescent Substrates

PhosphaGLO and PhosphaGLO Reserve AP Substrates were compared to CDP-Star® Chemiluminescent Substrate to demonstrate the relative signal provided when detecting proteins at low concentrations. Each substrate was used according to the manufacturer's recommended protocol. PhosphaGLO and PhosphaGLO Reserve showed greater sensitivity as demonstrated by the detection of lower protein concentrations than CDP-Star on both PVDF and nitrocellulose membranes. In addition, the background with PhosphaGLO was equivalent to that seen with CDP-Star. As a result, signal-to-noise ratios are significantly higher with PhosphaGLO AP Substrates.



**Figure 1:** Comparison of low-end sensitivity using PhosphaGLO Reserve AP Substrate, PhosphaGLO AP Substrate, and CDP-Star Chemiluminescent Substrate on nitrocellulose and PVDF membranes. Five-fold serial dilutions of Mouse IgG (2 ng - 3.2 pg) were separated by SDS-PAGE and transferred to the membranes. Protein was detected using a 1:1,000 dilution of biotin-labeled goat anti-mouse IgG, a 1:10,000 dilution of AP-labeled streptavidin and each respective substrate. CDP-Star was mixed with Nitro-Block-II™ Luminescence Enhancer before applying it to the nitrocellulose blot. Film was exposed for 10 minutes.

### Ordering Information

Catalog#	Description	Size
55-60-03	PhosphaGLO™ AP Substrate	30 mL
55-60-04	PhosphaGLO AP Substrate	100 mL
55-60-01	PhosphaGLO Reserve™ AP Substrate	30 mL
55-60-02	PhosphaGLO Reserve AP Substrate	100 mL

To order or for more information on KPL's full line of protein and nucleic acid detection products, contact us at 800.638.3167 / 301.948.7755, FAX 301.948.0169 or visit us at [www.kpl.com](http://www.kpl.com).

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CDP-Star Chemiluminescent Substrate is a registered trademark and Nitro-Block-II is a trademark of Applied Biosystems.

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### Frequently Asked Questions

**Q:** Will I need to use any special blocking agents in Western blotting?

**A:** Special blocking agents are not needed for use with either PVDF or nitrocellulose membranes. Use of Detector Block may improve your signal-to-background ratio. We have found greater sensitivity with PVDF than with nitrocellulose.

**Q:** How long are the substrates stable when stored at 4° C? Can they be stored frozen? Should they be stored in the dark?

**A:** Both substrates are stable for 2 years from date of receipt when stored at 4° C. They can be stored frozen but repeated freezing and thawing may degrade the substrates. As a result, freezing is not recommended. The substrates should be stored in the dark. Exposure to light for short periods of time will not harm the substrates.

**Q:** How does PhosphaGLO Reserve AP Substrate compare to premium HRP substrates such as LumiGLO Reserve™?

**A:** Detection limits for both products are in the femtogram range. Whereas LumiGLO Reserve will give you signal faster, PhosphaGLO Reserve allows you to capture sensitive images over a longer period of time. It gives you more versatility in your result exposures.



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