TECHNICAL SERVICE REPORT

Stability of LumiGLO Reserve™ HRP Chemiluminescent Substrate (Cat. No. 54-71-00, 54-71-01, 54-71-02)

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Purpose

To measure the stability and performance of the LumiGLO Reserve™ Chemiluminescent Substrate when stored at 2 – 8° C over time.

Materials and Methods

The following lots of material were tested. Representative bottles from each lot of A and B solution were stored at 4° C (recommended storage). Testing was completed in August 2010. The lot numbers tested are listed below.

Lot No.	Date of Manufacture	Age of Material
080364	03/05/2008	31 months
090048	10/07/2008	22 months
090745	06/17/2009	14 months
100120	02/17/2010	6 months
100549	08/01/2010	3 weeks

The samples were evaluated using Western Blotting followed by detection using KPL reagents.

1. A Bio-Rad SDS-PAGE system was used for the running and transfer of Mouse IgG titrated 1:2 in running buffer from 500 pg in lane one of the gel to 7.8 pg in the final 7th lane. All samples were heated to 95° C for 3 minutes prior to loading. Dilutions were run in duplicate sets on the gels in order to compare multiple lots of LumiGLO Reserve.

2. After transfer to nitrocellulose membrane, membranes were blocked at 4° C in 1X Detector Block with 1% Detector Block Powder.

3. The block was replaced with HRP-labeled, Goat anti-Mouse IgG (H+L) conjugate (KPL Catalog No. 374-1806) diluted 1:10,000 in 1X Detector Block (no powder). Blots were incubated at room temperature for one hour with shaking.

4. Blots were washed 3 x 5 minutes, followed by a final 15 minute wash using 1X Wash Solution (KPL Catalog No. 50-63-00). Blots were separated after this step.

5. LumiGLO Reserve HRP Chemiluminescent Substrate was added to the blots for one minute without shaking. Solutions and incubations were protected from light.

6. Blots were then lightly blotted with Whatman paper and placed in sheet protectors.

7. Blots were exposed to film for 10 minutes to determine the sensitivity relative to one another. Background was determined when all bands were observed.

Immunization

KPL prepares most of its affinity purified antibodies by using highly purified normal immunoglobulins as the injected antigen. The use of normal IgG, IgM or IgA as immunogen instead of monoclonal immunoglobulins from myeloma proteins yields an antiserum that has broad reactivity to all subtypes of the normal IgG, IgM or IgA. With few exceptions KPL also immunizes animals with complete antibody molecule (IgG) instead of the Fc fragment. Although this method necessitates further purification and lowers the yield, the resultant antibody is assured of reacting broadly with the target antigen.

Results

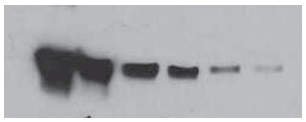
Lots of LumiGLO Reserve manufactured in 2008 (31 months and 22 months) showed 6 bands and those manufactured in 2009/2010 (14 months, 6 months and 3 weeks) showed 7 bands. Background was minimal with all lots tested.

Figure 1. Sensitivity Between Different Lots of LumiGLO Reserve Chemiluminescent Substrate.

Lot No.	Number of Bands	Limit of Detection
080364	6	15.625 pg
090048	6	15.625 pg
090745	7	7.8 pg
100120	7	7.8 pg
100549	7	7.8 pg

Lot # 080364

Lot # 100120



Lot # 090048

Lot # 100549

Lot # 090745

Conclusions

KPL's LumiGLO Reserve Chemiluminescent Substrate maintained high sensitivity with excellent background levels for a minimum of 31 months from date of manufacture when stored at 2 – 8° C. This is demonstrated via low picogram detection (less than 100 pg). We expect the detection limit to continue to decrease, and background levels to stay low over time.

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