Technical Service Report

Stability of LumiGLO® Chemiluminescent Peroxidase Substrate System

Purpose:

To evaluate the stability and lot-to-lot consistency of LumiGLO® Chemiluminescent Peroxidase Substrate System stored at 4°C and room temperature.

Reagents:

This study compares the performance of four lots of LumiGLO Chemiluminescent Peroxidase Substrate Solution A and Substrate Solution B. Representative samples of each of the following lots were stored at 4°C and room temperature.

Substrate A Lot #	Substrate B Lot #	Age of Solutions
PL34	PL35	33 months
QL34	QL35	21 months
RH15	RH16	12 months
SG16	SG13	1 month

Test Parameters:

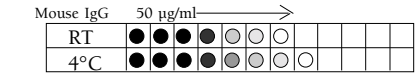
The components were evaluated using a dot ELISA test procedure performed on standard nitrocellulose (Schleicher & Schuell) as follows:

1. Prepare two-fold dilutions of Rabbit IgG (Cappel Lot 35600) in a microwell ELISA plate, starting at a concentration of 50 µg/ml in PBS.
2. Mark the membranes with a grid (Figure 1), using an appropriate pen.
3. Transfer 1.0 µl of the diluted Rabbit IgG from each well in the dilution plate to the appropriate spot on duplicate gridded membrane strips using a microdispenser. Air dry strips approximately 5 minutes to allow protein to adhere to the membrane.
4. Block strips with 0.5% Milk Diluent/Blocking Solution (Product Code 50-82-00), Lot QM14, for 1 hour at room temperature.
5. Incubate strips with Peroxidase-Labeled Goat Anti-Rabbit IgG (H+L), (Catalog No. 14-15-06), Lot RE58-5, diluted to 0.1 µg/ml in 0.5% Milk Diluent/Blocking Solution, for 1 hour at room temperature.
6. Wash strips with three 10 minute soak periods using Wash Solution Concentrate (Product Code 50-63-02), Lot PA05, diluted 1:20.
7. Prepare working solution of substrate by mixing equal volumes of Chemiluminescent Substrate A (Product Code 50-59-00) and Chemiluminescent Substrate B (Product Code 50-60-00).
8. Place strips in substrate solution. Incubate at room temperature for 1 minute.
9. Remove strips from substrate, blot excess liquid, and place strips between plastic sheets.
10. Expose strips to Kodak X-OMAT film for two minutes. Develop film and determine the amount of Rabbit IgG detected for each lot of substrate.

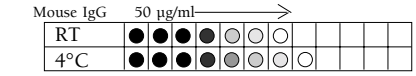
Results:

Figure 1.

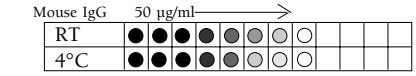
PL34 + PL35



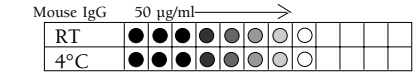
QL34 + QL35



RH15 + RH16



SG16+ SG13



Results:

All samples of LumiGLO Chemiluminescent Peroxidase Substrate detected Rabbit IgG in the range of 0.3-0.8 µg/ml. (Figure 1) The older lots of Solution A (PL34 and QL34), when stored at room temperature, became light yellow in color and produced a less intense reaction with approximately a two-fold decrease in sensitivity.

Conclusions:

KPL's LumiGLO Chemiluminescent Peroxidase Substrate Solution is very consistent in performance from lot to lot, and has no loss of sensitivity when stored at 4°C for up to 33 months or 1 month at room temperature.

ML-137-04

2 Cessna Court, Gaithersburg, MD 20879-4174 USA 301.948.7755 800.638.3167 FAX 301.948.0169