Technical Service Report

Comparison of LumiGLO® Chemiluminescent Peroxidase Substrate System to Brand 2

Purpose:

To compare the sensitivity and duration of signal obtained with KPL LumiGLO® Chemiluminescent Peroxidase Substrate System to results obtained with a competitve product.

Reagents:

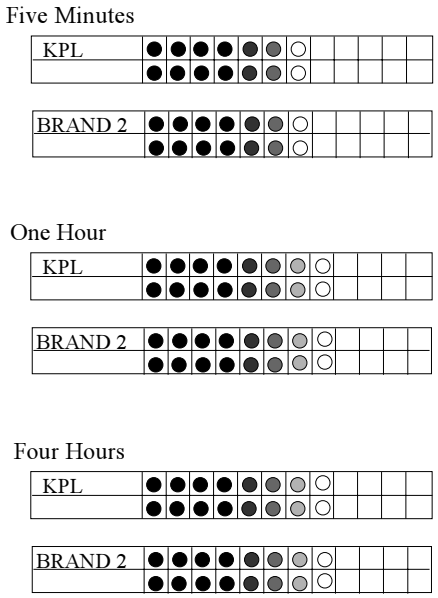
Solution A Lot No.	Solution B Lot No.	Manufacturer
PL34 Lot 5	PL35 Lot 5	KPL Brand 2

Test Parameters:

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

1. Prepare two-fold dilutions of Mouse IgG (Cappel Lot 35327) in a microwell plate, starting at a concentration of 25 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 Mouse IgG from each well in the plate to the appropriate spot on duplicate gridded membrane strips. Air dry strips approximately 5 minutes to allow protein to adhere to the membrane.
4. Block strips with 0.5% Milk/Diluent Blocking Solution (Catalog No. 50-82-01), Lot NJ03, for 30 minutes at room temperature.
5. Incubate strips with Peroxidase-Labeled Goat Anti-Mouse IgG(H+L), (Catalog No. 14-18-06), Lot NF26-5, diluted to 0.1 µg/ml in 0.2% Milk/Diluent Blocking Solution, for 30 minutes at room temperature.
6. Wash strips with three 10 minute soak periods using Wash Solution Concentrate (Catalog No. 50-63-00), Lot PA05, diluted 1:20.
7. Prepare working solution of both substrate brands by mixing equal volumes of Solution A and Solution B.
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 periods of five minutes, one hour, and four hours. Develop film and determine the amount of mouse IgG detected for each lot of substrate.

Figure 1



Results:

For both brands of chemiluminescent substrate, mouse IgG was detected to a concentration of 0.39 µg/ml after a five minute exposure. When strips were exposed for either one or four hours, mouse IgG was detected to a concentration of 0.195 µg/ml with both brands of substrate. (See Figure 1.)

Conclusion:

There is no difference in sensitivity or duration of signal emission between KPL LumiGLO® Chemiluminescent Peroxidase Substrate and the competitive brand tested.

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