KPL TECHNICAL SERVICE REPORT

Stability of LumiGLO® Ultra Chemiluminescent Substrate after Mixing

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Purpose

To evaluate the stability of KPL's LumiGLO Ultra Chemiluminescent Substrate after Solution A (Catalog No. 54-49-01, Lot No. 120226) and Solution B (Catalog No. 54-50-01, Lot No. 120227) have been mixed together in equal volumes and allowed to incubate in the dark at either ambient (20°C - 25°C) or cold (4°C - 8°C) temperatures for the indicated amount of time. The stability of the mixed solutions will be measured by comparing chemiluminescent signal over time in a directly detected ELISA.

Materials and Methods

1. Nunc 96-well MaxiSorp black plates (Catalog No. 475515) were adsorbed with serially diluted (1:2) Human IgG starting at 200 ng/mL (100 µL/well).

2. 100 µL of KPL's HRP labeled Anti-Human IgG (Catalog No. 04-10-06, Lot No. 100893) diluted in 1% BSA to 50 ng/mL was added to each well. Plates were incubated for 45 minutes at ambient temperature.

3. Plates were washed 6 times using a mechanical plate washer (Dynex Technology's Ultrawash PLUS) and KPL's 20X Wash Solution Concentrate (Catalog No. 50-63-19, Lot No. 050512) diluted 1:20 in pico-pure water.

4. 100 µL of mixed LumiGLO Ultra Chemiluminescent Substrate was added to each well. The plate was allowed to incubate for 5 minutes before determining the luminescent output using a PerkinElmer Victor^3 Plate Reader.

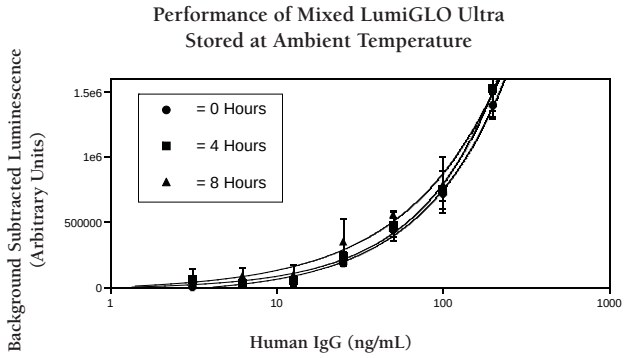
5. Luminescent intensities from individual wells were background subtracted and plotted versus the corresponding antigen concentration. Data points in the graphs are averages from 3 separate wells and error bars represent the standard deviation. Four-parameter fit curves were generated in the software program SoftMax Pro (Molecular Devices).

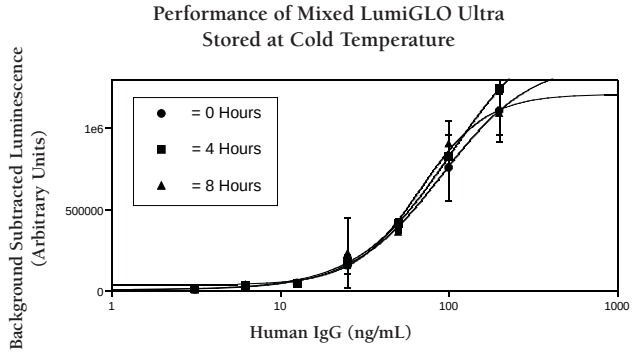
6. Performance of the substrate was measured by comparing identical chemiluminescent direct ELISAs at 0, 4, and 8 hours after initial mixing of the 2 solutions.

Time After Mixing	Mixed Storage Conditions
0 Hours	Ambient or Cold
4 Hours	Ambient or Cold
8 Hours	Ambient or Cold

Results

Performance of the mixed LumiGLO Ultra Chemiluminescent Substrate at 0, 4, and 8 hours were within error of each other at either ambient (20°C - 25°C) or cold (4°C - 8°C) temperature (see figures below) when measured via ELISA.





Conclusion

Mixed LumiGLO Ultra is stable for up to 8 hours at either ambient (20°C - 25°C) or cold (4°C - 8°C) temperatures. Sample must be protected from light during mixed storage.

Notes

KPL stability studies have shown a decrease in performance (diminished signal strength) of LumiGLO Ultra Chemiluminescent Substrate when the individual components, Solution A and Solution B, are exposed to temperatures of 37°C.

While it is appropriate to compare relative changes in performance over time on individual plates, absolute luminescent values may vary somewhat from plate to plate.

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