CellTiter-Glo® 2.0: A Novel Luminescent Cell Viability Assay with Greatly Enhanced Storage Stability

Michael P. Valley, James Unch, Poncho L. Meisenheimer, James J. Cali, and Dan F. Lazar

2800 Woods Hollow Road, Madison, WI 53711-5399, USA

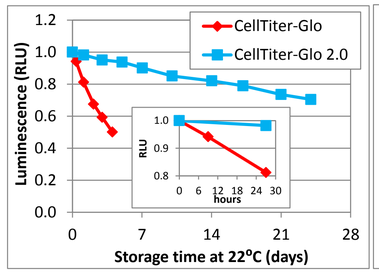
1. Introduction

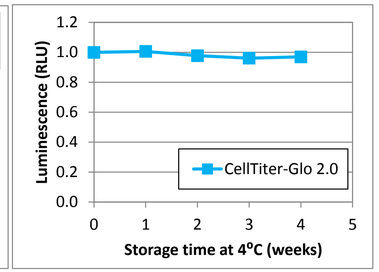
Here we report on the attributes of a novel ATP detection reagent for cell viability with all of the assay performance of the previous reagent, CellTiter-Glo®, but now with markedly enhanced stability as a single component in a liquid format. These new features provide for much greater ease-of-use in that storage of the reagent at 4°C eliminates the requirement for reagent thawing and minimizes temperature equilibration time. The enhanced stability also means that reagent performance is maintained at room temperature during extended assay operations, and any unused reagent can be retained for future use by simply returning it to 4°C storage rather than having to freeze or discard it.

2. Enhanced Stability

Samples of reagent were placed at 22C or 4C for different lengths of time and then frozen at -20°C. Once all samples were collected, they were thawed and assayed by mixing 1:1 with 2 µM ATP in water. Luminescence was read at 10 min.

	CellTiter-Glo	CellTiter-Glo 2.0
> 85% activity @ 22°C	12 hours	7 days
> 90% activity @ 4°C	3.5 days	4 weeks





3. Performance with various cells & media

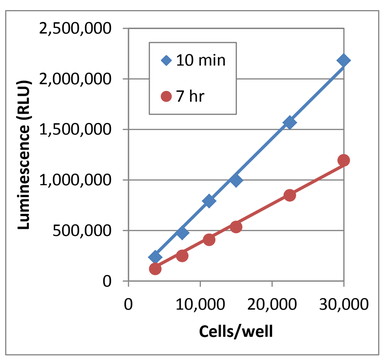
10,000 cells were plated for 24 hours, mixed 1:1 with reagent, and the luminescence was read over time.

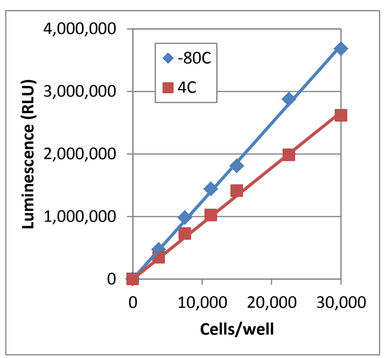
		Luminescence (RLU x 10^6) @ 10'		Signal Half-life (hr)	
cell type	media	CellTiter-Glo	CellTiter-Glo 2.0	CellTiter-Glo	CellTiter-Glo 2.0
MCF7	MEM	4.06	6.40	7.30	4.81
DU145	MEM	8.42	12.45	7.00	5.13
U2OS	McCoys 5A	5.98	9.27	7.14	5.07
CHO	F12	5.86	8.76	6.97	4.66
HEK293	DMEM	6.21	10.07	7.24	4.93
HeLa	DMEM	5.80	9.01	7.02	4.88
HepG2	DMEM	6.52	10.34	7.27	4.83
HCT116	RPMI	6.75	10.86	7.53	4.95
Jurkat*	RPMI	12.80	21.10	7.41	4.88
U937*	RPMI	13.51	20.86	7.07	5.33

*50,000 cells were plated for suspension cells.

4. Linearity over time

Jurkat cells were mixed 1:1 with CellTiter-Glo® 2.0. The left graph shows luminescence of samples assayed with the same reagent at 10 min and 7 hr. The right graph shows luminescence of samples at 10 min with reagent stored for 4 months at 4°C or -80 °C.





5. Implications of the new format

In contrast to other ATP detection reagents that are available as separate containers of substrate and buffer, CellTiter-Glo® 2.0 will be available as a liquid in a single container. Such a format change is possible due in part to the greatly enhanced stability of this new reagent, and hence there are a variety of storage options.

- If the reagent is to be used within a few months, it can be stored at 4°C. This eliminates any thawing requirements prior to use.
- Storage at 4°C also means that pre-equilibration to room temp is easier. The reagent need only be placed in a room temperature water bath for a short period of time or it can simply be left out at room temperature the day before use.
- And if the reagent is not intended to be used for several months, it can be stored at -20°C for several years.

6. Conclusion

This novel luminescent cell viability assay has three key features.

- Greatly enhanced reagent stability
- Performance similar to CellTiter-Glo®
- Single container liquid format

CellTiter-Glo® 2.0 will find great value not only with scientists looking to do screening runs over several days but also with scientists who simply wish to be able to store their viability reagent above freezing temperatures.