Fast Post-thaw Recovery of Cryopreserved Cells for Use in Bioassays

Lukas Underwood, PhD; Weiguo Shu, PhD; Rajnee Kanwal, PhD; Diana Douglas, BS; Joshua Franklin, BS; Jacqueline Mikhaylov, BS; Nilay Chakraborty, PhD
ATCC, Manassas, VA 20110 | Email: lunderwood@atcc.org

Background

- Large-scale bioassays often used for drug screening, vaccine development, and quality control require standardization, reproducibility, and automation compatibility for success.
- The large-scale cell culture of the biological material inputted into these assays poses the greatest challenge for success.
- The time and resources required to produce sufficient biomaterial as well as additional risks such as genetic drift, phenotypic changes, and contamination can make development and sustainability of a bioassay difficult.
- High content, highly standardized cell material in a cryopreserved format could replace the need for risky large-scale culture if post-thaw characteristics are comparable to those in live culture.

Materials and Methods

- The post-thaw viability, growth, and functional characteristics of a cryopreserved THP-1 luciferase reporter cell line (ATCC® TIB-202-NFkB-LUC2™) were evaluated and compared to a culture control.
- A THP-1 luciferase reporter derivative was cryopreserved in a proprietary fetal bovine serum (FBS)–free cryoformulation.
- Viability and growth were evaluated using a trypan blue–based automated counting instrument.
- The luciferase reporter cell line expresses bioluminescent proteins in response to stimuli activating NFkB pathways. *E. coli* lipopolysaccharide (LPS) was used for reporter stimulation.

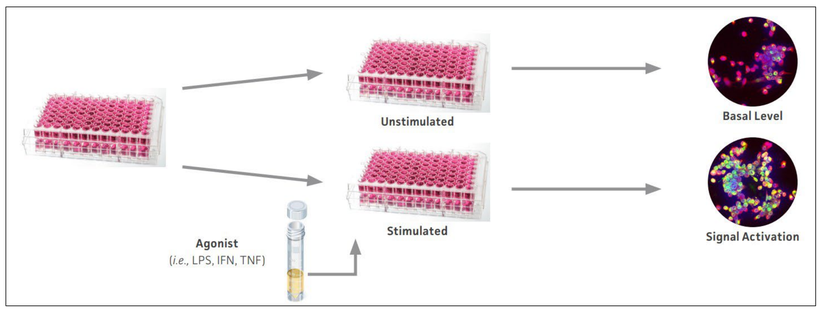

Figure 1: Quantitation of immunomodulation using NFkB reporter cells

Post-thaw Viability

Batch and Sample	Post-thaw Viability	Viable Cells per Vial	Yield
Batch 1 - Sample 1	95.0%	14,251,000	95.0%
Batch 1 - Sample 2	95.7%	14,189,000	94.6%
Batch 1 - Sample 3	95.9%	14,288,000	95.3%
Batch 1 - Sample 4	93.9%	13,645,000	91.0%
Batch 2 - Sample 1	93.4%	14,898,000	99.3%
Batch 2 - Sample 2	92.9%	13,991,000	93.3%
Batch 2 - Sample 3	94.8%	14,942,000	99.6%
Batch 2 - Sample 4	93.6%	13,002,000	86.7%

Table 1: Post-thaw cell characteristics. Four samples from two separately cryopreserved batches were thawed and evaluated. Viability is a percentage determined by the ratio of live cells over total cells counted. The third column records the total viable cells determined by multiplying average viable cells per image by a scaling factor. Yield is a percentage calculated by the ratio of live cells recovered over the live cells initially vialed (15 million cells).

Post-thaw Growth and Functionality

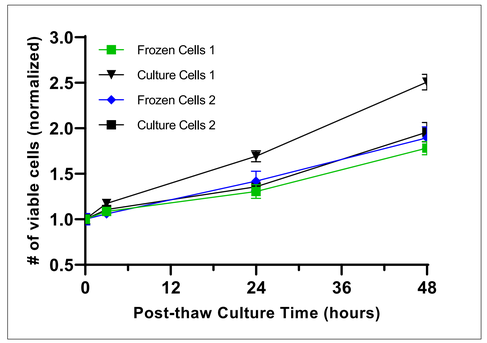

Figure 2: Comparison of post-thaw growth. Growth of live cells was determined by normalizing all counts to the count taken immediately after thawing. The growth of two separate batches of frozen-thawed cells were compared to two separate batches of cells grown in culture for several weeks. Interestingly, the two frozen samples had similar growth kinetics to that of the second culture sample while culture sample 1 grew much faster. On average, freshly thawed cells typically grow slower, but have less variability compared to cells in culture. n=4, ±SEM.

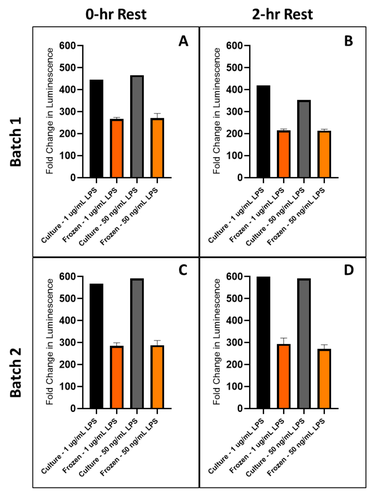

Figure 3: Evaluation of post-thaw cellular luminescence. Both frozen and culture control samples were evaluated for luminescence following a 3-hour stimulation with LPS (1 µg/mL or 50 ng/mL). The cells were either tested immediately (0-hr rest) or allowed 2 hours of rest. Two separate batches of cells were tested for comparison. Similar trends are observed for all figures. The thawed samples always have less luminescence than culture control, between 200-300-fold change. There is minimal effect observed between post-thaw hold times and concentration of LPS. Like growth kinetics, we observe higher variability between batches for the culture control cells. n=3, ±SEM.

Shipping Evaluation

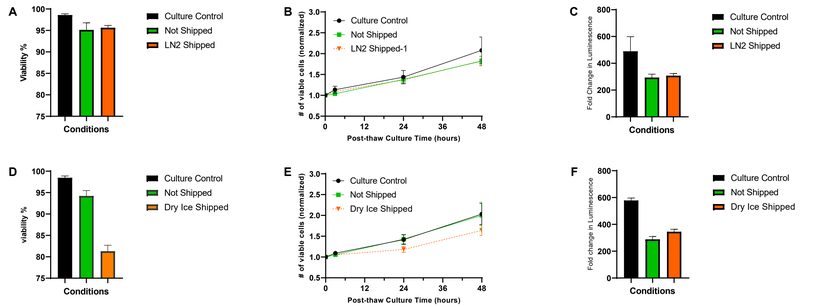

Figure 4: Evaluation of the effect of shipping on the post-thaw cell characteristics. While we observe strong post-thaw recovery of the cells pulled from internal storage, it is important to note the biggest risk of storage temperature deviations is during shipment of the cells. Here, two shipment conditions were studied: (A-C) LN_2 shipment and (D-F) dry-ice shipment. (A,D) The post-thaw viability of samples shipped was compared to that of the not shipped and culture control conditions. Only cells shipped in the dry-ice condition had reduced post-thaw viability. A similar trend is observed when evaluating (B,E) growth kinetics. The cells shipped on dry ice have a longer lag phase post-thaw. Despite the reduced viability and growth observed, the (C,F) luminescence response was unaffected by the shipping conditions. n=6, ±SEM.

Supplementary – Luminescence Optimization

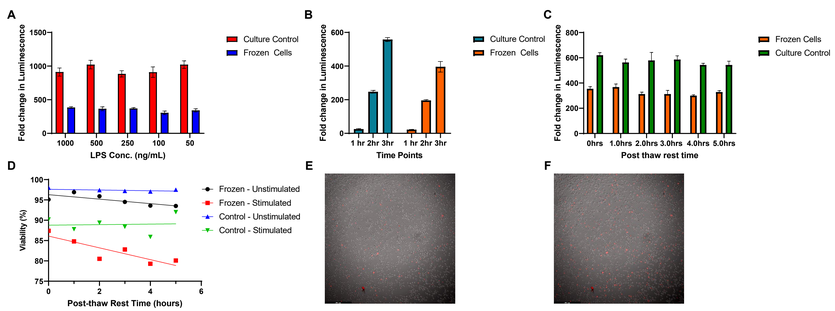

Figure 5: Luminescence evaluation. There are several variables that can be altered that effect the outcome of the luminescence data. The use of LPS for stimulation is kept consistent; however, it should be noted that several immunogenic molecules can be used. (A) LPS concentrations between 50–1000 ng/mL had a minimal effect on luminescence response when stimulated for 3 hours. (B) There is major correlation between stimulation time and luminescence response; however, this begins to plateau after 3 hours (data not shown). n=6, ±SEM. (C,D) There is a subtle decrease in luminescence as post-thaw rest time increases. This is strongly correlated to a reduction in post-thaw viability with time. Additionally, LPS stimulation causes cell death resulting in ~10% drop in viability. (E,F) Before and after photos of LPS stimulation on freshly thawed cells stained with ethidium bromide dye. Red cells indicate dead-cells with permeable cell membranes.

Conclusions

- Cryopreserved cells have high post-thaw viability (>90%) and yield. Growth and immediate post-thaw luminescence, while slightly lower, are more consistent compared to the culture control.
- There is some concern about maintaining strong post-thaw viability and growth of the cells after dry-ice shipping.
- Further evaluation is required to determine the root cause of lower recovery and to evaluate whether prevention is possible.

ATCC 10801 University Boulevard, Manassas, Virginia 20110-2209 **Phone:** 800.638.6597 **Email:** licensing@atcc.org **Web:** www.atcc.org