RNA-Seq Analysis Confirms Post-Thaw Transcriptomic Stability in ThawReady™ THP-1 Assay-Ready Cells

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Abstract

Cryopreserved cell lines have become essential for high-throughput screening and assay development, offering convenience and consistency across experiments. However, the freeze–thaw process can induce cellular stress, potentially affect gene expression, and compromise functional reliability. To ensure consistent assay performance, it is important to confirm that cryopreserved cells retain their transcriptomic and functional integrity following thawing.

In this study, we evaluated the transcriptomic stability of ThawReady™ THP-1 (ATCC® TIB-202-AR™) cells using RNA sequencing (RNA-seq). Cells were analyzed immediately after thawing (0-hour) and following 2-hour and 8-hour recovery intervals and were compared to freshly cultured THP-1 cells. Principal component and hierarchical clustering analyses revealed that post-thaw samples closely grouped with fresh controls, demonstrating preservation of cellular identity and global gene expression patterns. Gene expression profiles showed strong concordance across key immune and inflammatory pathways characteristic of THP-1 cells.

Differential expression analysis identified 178 genes altered at 0 hours, 951 at 2 hours, and 713 at 8 hours post-thaw relative to fresh culture, using threshold of absolute fold change >5 and FDR-adjusted p-value <0.05. Despite these transient changes, pathways related to cell survival and proliferation remained stable. Notably, phagosome formation was the top enriched pathway, suggesting adaptive recovery responses that support cell viability.

Overall, these results confirm that ThawReady™ THP-1 cells maintain robust transcriptomic integrity post-thaw, supporting their reliability and reproducibility for downstream immune and inflammation-related functional assays.

Introduction

- THP-1 (ATCC® TIB-202™) – THP-1 is an authenticated human monocytic cell line that was originally derived from the peripheral blood of a patient with acute monocytic leukemia. These cells are widely used as an immune-relevant in vitro model because they retain key monocyte/macrophage characteristics, including cytokine responsiveness, phagocytic capability, and expression of inflammatory pathway genes. Their reproducible behavior makes them a foundation for host-response, immunology, and inflammation studies.
- ThawReady™ THP-1 (ATCC® TIB-202-AR™) – The ThawReady™ THP-1 cell line is a next-generation format of THP-1 cells that are pre-qualified and cryopreserved in an assay-ready state. These cells are optimized for immediate use post-thaw with minimal recovery time, enabling high-throughput screening and consistent assay performance. Despite freeze–thaw stress, ThawReady™ THP-1 cells are preserved to maintain stable transcriptomic and functional profiles, supporting reliable and reproducible immune-related assays.

Materials and Methods

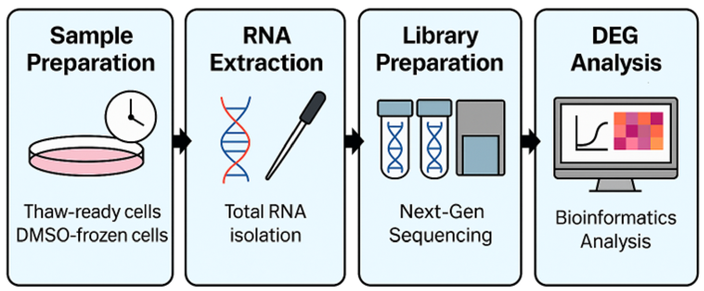

Figure 1: RNA-seq experimental workflow for THP-1 and ThawReady™ THP-1 cells. This schematic outlines the RNA-sequencing (RNA-seq) workflow used to characterize transcriptional differences between THP-1 and ThawReady™ THP-1 cells. ThawReady™, DMSO-frozen cells from both lines were recovered and sampled at 0 hours, 2 hours, and 8 hours post-culture recovery, with an additional control sample collected after 6 days of post-thaw culture to represent fully recovered cells. Total RNA was extracted using the QIACube automated protocol (QIAGEN), with samples meeting a minimum RNA integrity threshold of RIN > 6.5. Purified RNA was then used to generate sequencing libraries, which were processed on the Illumina NextSeq 2000 platform to obtain high-quality next-generation sequencing data. Sequencing reads were analyzed through the CLC Genomics Workbench pipeline to perform transcript quantification and identify differentially expressed genes between THP-1 and ThawReady™ THP-1 cell populations.

Results

Principal component analysis reveals distinct transcriptional separation between ThawReady™ THP-1 and THP-1 across recovery timepoints

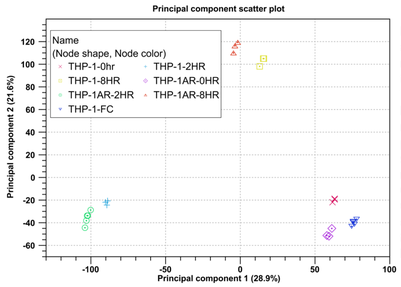

Figure 2: Principal component analysis (PCA) of THP-1 and ThawReady™ THP-1 transcriptomes across post-thaw recovery timepoints. This PCA scatter plot illustrates global transcriptional variation among THP-1 and ThawReady™ THP-1 (THP-1AR) cells collected at 0 hours, 2 hours, and 8 hours after post-thaw culture recovery, along with fully recovered 6-day cultured control samples (THP-1-FC). Each point represents an individual RNA-seq sample, with node shape and color corresponding to specific cell types and recovery timepoints as indicated in the legend. Principal Component 1 (28.9% variance explained) and Principal Component 2 (21.6% variance explained) together highlight clear clustering patterns, demonstrating distinct transcriptional trajectories over early recovery and robust separation between THP-1 and ThawReady™ THP-1 populations.

Comparative RNA-seq identifies differentially expressed genes between ThawReady™ THP-1 and THP-1 cells

A

	THP-1AR
Time Point	DEG
0-hr PT	178
2-hr PT	951
8-hr PT	713

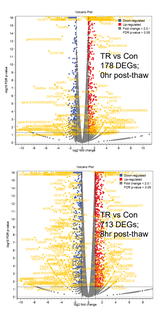

Figure 3: Transcriptomic profiling of ThawReady™ THP-1 post-thaw reveals dynamic gene expression changes. (A) Summary table showing the number of differentially expressed genes (DEGs) in ThawReady™ THP-1 cells relative to 6-day fresh THP-1 cultures across post-thaw recovery time points. Differential expression analysis was performed in a time-resolved manner to capture early and intermediate transcriptional responses following thawing. (B–D) Volcano plots illustrating global gene expression changes in ThawReady™ THP-1 cells compared to day-6 fresh THP-1 reference samples at three post-thaw intervals. (B) Immediate post-thaw analysis identified 178 DEGs, representing rapid transcriptional perturbations. (C) At the 2-hour recovery time point, the number of DEGs markedly increased to 951, indicating a robust intermediate cellular response. (D) By 8 hours post-thaw, 713 DEGs remained significantly altered, suggesting partial normalization yet persistent transcriptional differences. Collectively, these analyses highlight substantial and dynamic time-dependent transcriptomic remodeling in ThawReady™ THP-1 cells immediately after thawing and during early culture recovery.

Time-dependent pathway changes in ThawReady™ THP-1 cells after thawing (IPA)

A

ThawReady™ Post Thaw 0-hour Ingenuity Canonical Pathways	-log(p-value)	Ratio	z-score
Phagosome Formation	9.17	0.0297	1.886
Role of Osteoclasts in Rheumatoid Arthritis Signaling Pathway	7.45	0.0411	1.265
S100 Family Signaling Pathway	6.94	0.0243	0
G alpha (i) signaling events	6.53	0.0426	0.302
Class A/1 (Rhodopsin-like receptors)	6.31	0.0361	1.155
ThawReady™ Post Thaw 2-hour Ingenuity Canonical Pathways	**-log(p-value)**	**Ratio**	**z-score**
S100 Family Signaling Pathway	10.9	0.0831	-3.615
Molecular Mechanisms of Cancer	10.4	0.079	-2.109
Phagosome Formation	10.1	0.0836	-1.089
Class A/1 (Rhodopsin-like receptors)	8.72	0.105	-1.183
G alpha (i) signaling events	8.56	0.116	-1.095
ThawReady™ Post Thaw 8-hour Ingenuity Canonical Pathways	**-log(p-value)**	**Ratio**	**z-score**
S100 Family Signaling Pathway	13.8	0.0767	-2.06
Granulocyte Adhesion and Diapedesis	12.5	0.137	#NUM !
Class A/1 (Rhodopsin-like receptors)	12	0.105	-0.845
G alpha (i) signaling events	10.8	0.112	-0.557
Hepatic Fibrosis/Hepatic Stellate Cell Activation	10.7	0.13	#NUM !

B

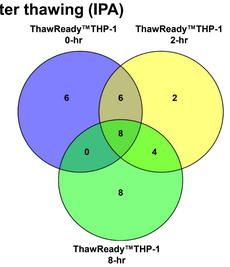

Figure 4: Pathway enrichment analysis of differentially expressed genes in ThawReady™ THP-1 cells. (A) Top enriched canonical pathways identified at each post-thaw analysis time point. (B) Venn diagram showing shared and unique pathway signatures across the analyzed time points. The Z-score indicates the strength of the association between a gene or protein and a pathway. A #NUM z-score suggest that the gene is not significantly associated with the pathway, meaning may not play a major role in the processes being analyzed.

Top differentially regulated gene sets and upstream regulator networks in ThawReady™ THP-1 cells

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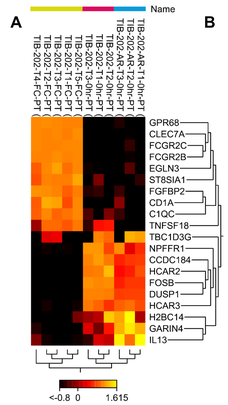
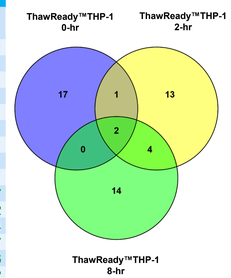


B

Gene	0-hr Fold change	Gene	2-hr Fold change	Gene	8-hr Fold change
FCGR2C	72.722	BIVM-ERCC5	1720.99	JPH2	127.007
FCGR2B	71.423	ST8SIA1	579.492	FRG2	101.912
ST8SIA1	43.922	TNFRSF6B	402.273	FCGR2C	100.783
FGFBP2	31.675	FCGR2C	142.224	FUT6	92.376
C1QC	29.749	FCGR2B	91.199	FBN3	75.433
TNFSF18	25.354	GIMAP4	52.493	FAM135B	57.218
CD1A	23.807	FRG2	52.356	FCGR2B	54.94
EGLN3	23.13	CD1E	50.034	RIPOR2	46.491
GPR68	21.689	CD163	39.652	RGL4	41.171
CLEC7A	20.621	GPR82	37.921	CSN1S1	39.784
TBC1D3	-597.501	CELF3	-14075.5	SYT5	-698.827
GARIN4	-249.556	PNMA8C	-11123.7	TPPP3	-353.542
DUSP1	-48.375	CFAP126	-3118.74	CACNG4	-265.874
CCDC184	-46.151	FOXJ1	-2392.08	CFAP276	-240.457
FOSB	-45.333	CFAP276	-2074.92	NUP62CL	-222.216
HCAR3	-23.097	NUP62CL	-1826.06	CCN3	-219.562
IL13	-22.535	SYT5	-1815.52	C7orf57	-168.911
NPFFR1	-20.581	CFAP210	-1471.5	S100A16	-160.514
HCAR2	-18.481	CHST1	-1335.19	MAPK15	-155.593
H2BC14	-18.112	CCDC160	-1279.95	CEMIP	-142.65

C



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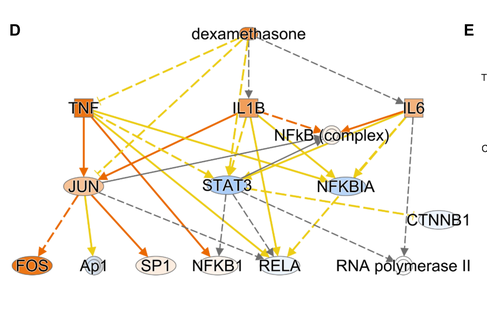

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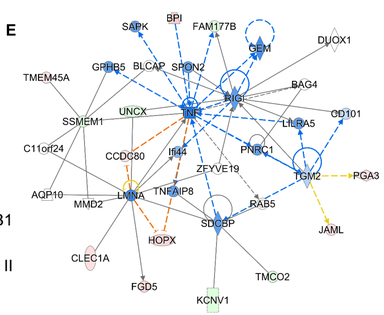

Figure 5: Gene set enrichment and upstream regulator analysis in ThawReady™ THP-1 cells. (A) Heatmap showing the top gene sets differentially expressed in ThawReady™ THP-1 and THP-1 cells relative to DMSO-treated, frozen THP-1-FC day-6 controls. (B) Table summarizing the most strongly up- or down-regulated gene sets, including fold-change values in ThawReady™ THP-1 versus THP-1 cells at the indicated time point. The identified gene set demonstrated consistent detection across all post-thaw interval analyses, with the intervals represented by different color annotations for clarity. (C) Venn diagram depicting shared and unique gene sets enriched in ThawReady™ THP-1 cells across the analyzed time point. Upstream regulator analysis identifying drivers of transcriptional changes in ThawReady™ THP-1 cells. (D) Network map of genes regulated by dexamethasone-associated signaling. (E) Network map highlighting upstream regulators connected to pathways involved in cell death and survival. FC: fully recovered 6-day cultured control samples.

Conclusions

- PCA and hierarchical clustering analyses showed that post-thaw ThawReady™ THP-1 samples clustered closely with fresh controls at the immediate post-thaw (0-hour) time point, indicating that cellular identity and overall transcriptomic architecture were largely preserved following thawing.
- Core immune and inflammatory gene signatures characteristic of THP-1 cells remained highly consistent between thawed and fresh cells.
- Differential expression analysis identified178 DEGs at 0 hours, 951 DEGs at 2 hours, and 713 DEGs at 8 hours using thresholds of fold change > 5 and FDR < 0.05.
- Although transient gene-expression changes were observed following thawing, pathways associated with cell survival, proliferation, and innate immune function showed no significant perturbation across recovery time points. These pathways were neither represented among differentially expressed genes nor enriched in IPA analyses, suggesting that core cellular and innate immune programs remained largely stable during early post-thaw recovery.
- Phagosome formation was identified as the top enriched pathway after thawing, indicating that ThawReady™ THP-1 cells engage innate recovery mechanisms that may contribute to sustaining cell viability during early post-thaw adaptation.
- While some transient changes are observed immediately post-thaw, ThawReady™ THP-1 cells largely preserve their transcriptomic integrity, supporting their reliability, reproducibility, and suitability for downstream immune- and inflammation-related functional assays.

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