Draft Report - Study# 218-001
Prepared by: Genemarkers

Cytotoxicity, Proliferation, Lipid Content, and Gene Expression Analysis of *In Vitro* Human Adipocytes Treated with Test Materials

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Approved by: Anna Langerveld (Mar 27, 2026 09:32:54 EDT) **Date:** 27-Mar-2026

Project Summary

The study was conducted to determine how the Sponsor's Test Materials (TMs) influence cytotoxicity, proliferation, lipid content and gene expression in adipocytes. An MTT assay was performed on pre-adipocytes treated for 24 hours with the TMs to determine the effects on cell viability/proliferation. An additional group of cells were grown in parallel and induced to form mature adipocytes. Fully differentiated, mature adipocytes were treated with the TMs for 24 hours. These cells were stained with Oil-Red-O to quantify lipid content and processed for qPCR analysis to determine the expression of select genes.

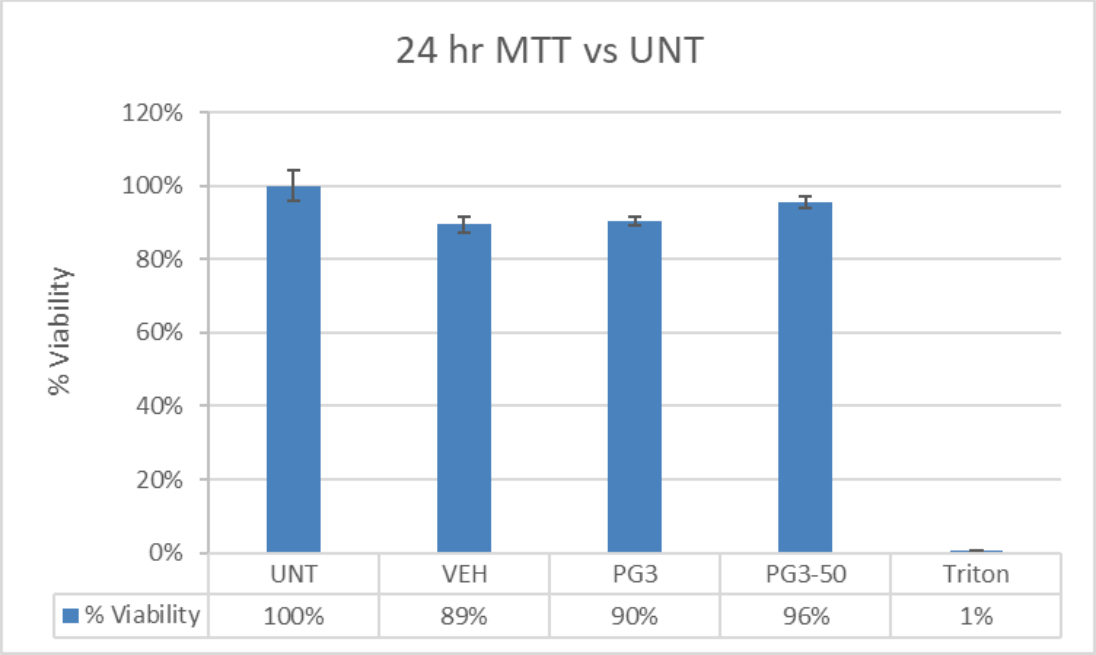
The following treatment groups were included in the study:

1. Untreated
2. Vehicle
3. PG3 - 0.001%
4. PG3-50 - 0.005%

Results: Cell Viability Analysis

Cell viability and proliferation was assessed using an MTT assay. Cells treated with Triton X-100 were used as the negative control. Viability was assessed following a 24-hour treatment with the TMs. Statistics (unpaired t-tests, $p<0.05$; N=3) were performed to determine statistically significant differences. There were no statistically significant changes in cell viability; the cells in the TM groups showed viability levels $\geq$89% when compared to the Untreated Control group (Figure 1).

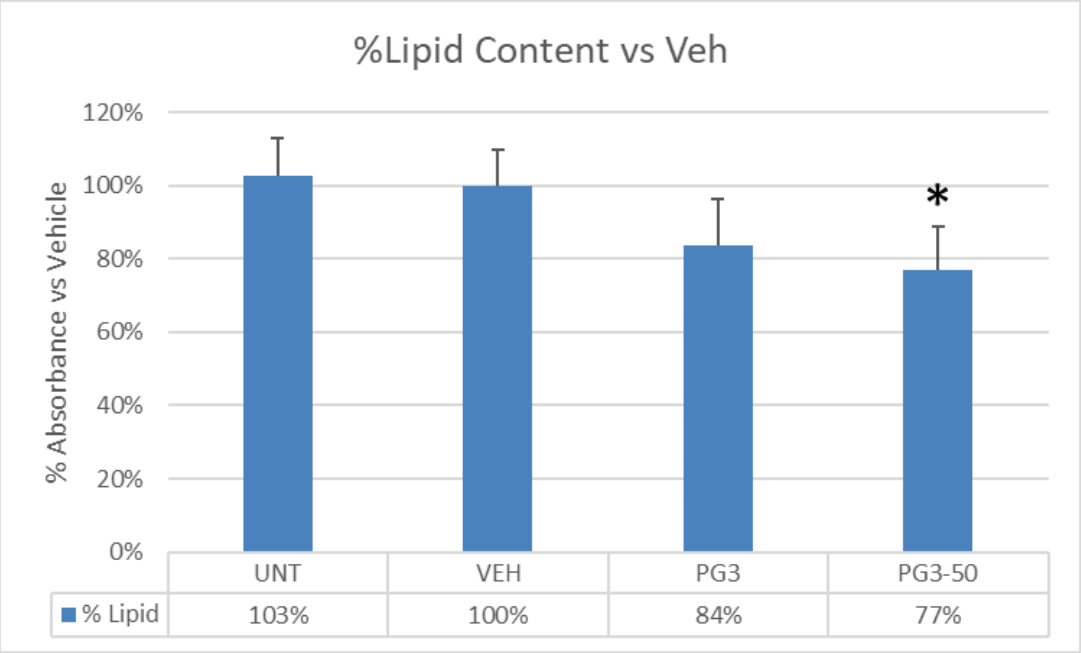
Figure 1. % Cell Viability/Proliferation Relative to the Untreated Control Group



Results: Lipid Content Assay

Lipid content was measured by staining the cells with Oil-Red-O and reading the absorbance at 492nm using a colorimetric plate reader. Oil-Red-O is a fat-soluble diazo dye used to stain neutral triglycerides and lipids. Statistics were performed to compare the TM groups with the Vehicle Control group. PG3-50 0.005% significantly reduced lipid content relative to the Vehicle control (Figure 2). PG3 0.001% also showed a reduction in lipid content, though this decrease was not statistically significant.

Figure 2. Percent Lipid Content Relative to the Vehicle Control Group



**p<0.05 vs. vehicle control*

Results: Gene Expression

Changes in gene expression for select genes were measured using qPCR. Statistics (unpaired t-test, $p<0.05$, N=4) were performed to compare the TMs groups with the Vehicle Control group. Changes in gene expression are shown below in Table 1. Not statistically significant results are listed as "n.s." in gray text. Genes are sorted alphabetically by Gene ID. Percent change is provided as an additional way to view the data. Linear fold-change values of 2 or greater are typically considered biologically relevant, but in the personal care industry, fold-change values of 1.5 are often seen in marketing materials.

ACACA, ADIPOQ, and SCD1 showed statistically significant increases in expression, although all changes were below 1.5-fold.

Table 1. Gene Expression Relative to Vehicle Control

Target Name	vs. VEH			
	PG3 0.001%		PG3-50 0.005%	
	Linear FC	% Change	Linear FC	% Change
ACACA	1.21	21%	1.18	18%
ACACB	n.s.	n.s.	n.s.	n.s.
ADIPOQ	1.31	31%	1.22	22%
FASN	n.s.	n.s.	n.s.	n.s.
SCD1	1.33	33%	1.19	19%
SREBF1	n.s.	n.s.	n.s.	n.s.

Conclusions:

- Viability:
  - Exposure to the TMs did not significantly impact cell viability or proliferation in the pre-adipocytes as measured by MTT assay.
  - At the tested concentrations and exposure time point, the TMs do not appear to be cytotoxic to preadipocyte cells, and they do not negatively affect cell proliferation when compared to untreated cells.
- Lipid Content/Oil Red O:
  - PG3-50 0.005% significantly reduced lipid content relative to the Vehicle Control.
  - PG3 0.001% also showed a reduction in lipid content, though this decrease was not statistically significant.
- Gene Expression:
  - The changes in gene expression observed in this study had fold change values less than 1.5, which may not translate into a significant biological effect. The data supports that the materials have an effect on the genes measured. It may be beneficial to evaluate different concentrations or time points in an additional study.
    - ACACA and SCD1 were slightly upregulated by both TMs; these genes are both involved in fatty acid biosynthesis.
    - ADIPOQ was also slightly upregulated by both TMs; upregulation of ADIPOQ has been shown to promote fat loss.

The following data is provided:

- 218-001 MTT Analysis 26MAR2026 SLC
- 218-001 Oil Red O Analysis 26MAR2026 SLC
- 218-001 Gene Expression Analysis 26MAR2026 SLC

Experimental procedures

Test Materials: Two test materials were received at Genemarkers on February 12th, 2026. The materials were labeled “SLB 001 PG3 (50%)” and “SLB 001 PG3”. Materials were stored at 4°C, protected from light, until use. TMs were prepared to their final concentrations using the appropriate cell culture media as described below and outlined in the Study Proposal.

Cell Culture: Preadipocytes were seeded into multi-well culture plates; preadipocytes were analyzed via MTT assay for proliferation/viability after 24 hours exposure to test materials. Additional preadipocytes were plated and allowed to differentiate into mature adipocytes for both Oil Red O staining and Gene Expression analysis. Preadipocytes were induced ~8 days post seeding by the addition of differentiation media comprised of phenol red free basal media (ScienCell, cat# 7211, Lot # 43145) with the addition of FBA (ScienCell, Cat # 0025, Lot # 42868), Dexamethasone (Sigma, Lot # 102758121), IBMX (Sigma), Insulin (Sigma, Lot # 0000475185), Biotin (Mp Bio, Lot # V11250P4175-1), and Pantothene (Sigma, Lot # 102776714) supplements. Adipocyte medium was changed every 4-5 days pre- and post-induction until 80% confluence and the appearance of lipid droplets indicated mature adipocytes.

Test Media Preparation: Test medium was prepared using the culture media described above with the addition of the test material compound(s)at the appropriate concentrations as outlined in the Study Proposal relevant for each treatment condition.

Cell Culture Treatment: At confluence, the cell culture media was replaced with fresh media containing the TMs. Four biological replicates were included per treatment group for Oil Red O and Gene Expression analysis; three biological replicates were included per group for MTT analysis. Following the test media addition, the plates were returned to the incubator at 37°C with 5% CO2 and ~95% relative humidity for 24-hours.

MTT Viability Assay: An MTT assay was used to assess cell viability (N=3 per treatment group); viable tissues will convert MTT into a blue/purple formazan salt that is detected by measuring absorbance at a specific wavelength (570nm) of light (A570). Following the removal of TM media, MTT medium (DMEM) containing 0.5mg/mL MTT powder (Sigma-Aldrich) was added to each well. Cells were incubated with MTT media for 2.5 hours. Following incubation, the MTT solution was removed from each well and the plate was blotted on a sterile Kimwipe to remove any excess MTT medium, and the wells were filled with 0.4mL of extractant isopropyl alcohol. Extraction plates were then sealed with tape to limit evaporation. The cultures were incubated in the extractant solution for 5 minutes on a plate shaker.

Following extraction, 50 µL aliquots of extract were transferred into duplicate wells of a 96-well microtiter plate. Absorbance @ 570nm was measured using a MultiSkan FC instrument (Thermo Scientific); fresh isopropyl alcohol was measured as a blank, with the mean absorbance of the blank subtracted from the other sample readings. Cell viability for each culture was calculated by comparing

the A570 reading of the test cultures to the A570 of the untreated control cultures, using the following formula:

% Viability = [Treated A570 / Untreated A570] * 100

Oil Red O Assay: Upon collection, the cells were washed twice with PBS and then fixed with 1 mL of 10% NBF for 1 hour at room temperature. After fixation, the cells were pre-washed with 1 mL of 60% isopropanol for 5 minutes at room temperature and then allowed to air dry. Cells were then stained with 1 mL Oil Red O Staining solution for 30 minutes. After the 30-minute incubation with the staining solution, cells were washed 5 times with dd-H2O. Microscopic images were taken, water was removed, and the cells were allowed to air dry. Once dried, dye was eluted using 1 mL 100% isopropanol. 200 uL of eluted isopropanol per sample was transferred to a clear flat-bottom 96 well plate for analysis. Absorbance @ 492nm was measured using a MultiSkan FC instrument (Thermo Scientific); fresh isopropyl alcohol was measured as a blank, with the mean absorbance of the blank subtracted from the other sample readings. Relative lipid content was calculated by comparing the A492 reading of the test cultures to the A492 of the vehicle control cultures, using the following formula:

% Lipid Content = [Treated A492 / Untreated A492] * 100

RNA: Total RNA Isolation was performed using a Qiagen RNeasy Mini Kit (Qiagen Lot# 58103352) and QIAzol Reagent (Qiagen Lot# 57801182), recommended for lipid-containing samples. RNA concentration and purity were determined using a Nanodrop 2000 spectrophotometer. All samples showed high-quality RNA metrics (Table 2).

cDNA Synthesis & Preamplification: cDNA was generated from RNA using a High-Capacity cDNA Reverse Transcription kit (Thermo Fisher).

qPCR Processing: qPCR reactions were run using validated Taqman® gene expression assays in a 384-well plate format. Assays were run in a Life Technologies QuantStudio 12K Flex instrument. Each gene was assayed in duplicate.

Data Analysis: qPCR data quality and statistical analysis was assessed and performed on the raw data files using ThermoFisher Connect Software (Life Technologies). Statistical analysis was performed using the relative quantitation (RQ) method. In the first step of an RQ analysis, the Cq value of the target gene is normalized to the Cq value of an endogenous control gene to generate the delta Cq (dCq). dCq values are calculated in order to normalize for variability between the samples that may occur during the experimental procedures. See the Endogenous Control Gene Selection section for more details.

Statistical Data Analysis Using ThermoFisher Connect Software: Unpaired t-tests were carried out using ThermoFisher Connect software. The statistical comparison generated delta delta Cq [dd Cq] values (the mean dCq of the treated group – the mean d Cq of the control group). The statistical software converts the

dd Cq values into log and linear RQ values for export [RQ = 2^{-ddCq}]. The linear RQ values were converted to linear fold-change values to simplify data interpretation; linear fold-change data was calculated from exported linear RQ values using Microsoft Excel:

- For RQ values $\geq$1.0: Linear fold-change value = RQ value
- For RQ values <1.0: Linear fold-change value = -1/RQ value

The Excel file includes qPCR data shown with p-values, linear RQ values, and linear fold-change values.

RNA Quality

Sample concentration and purity were determined using a Nanodrop spectrophotometer. A260/280 readings indicate sample purity with ideal measurements that range from 1.8-2.1. The A260/230 ratio is an additional measure of sample purity. All samples showed high-quality RNA metrics (Table 2).

Table 2. RNA Sample Quantity and Quality

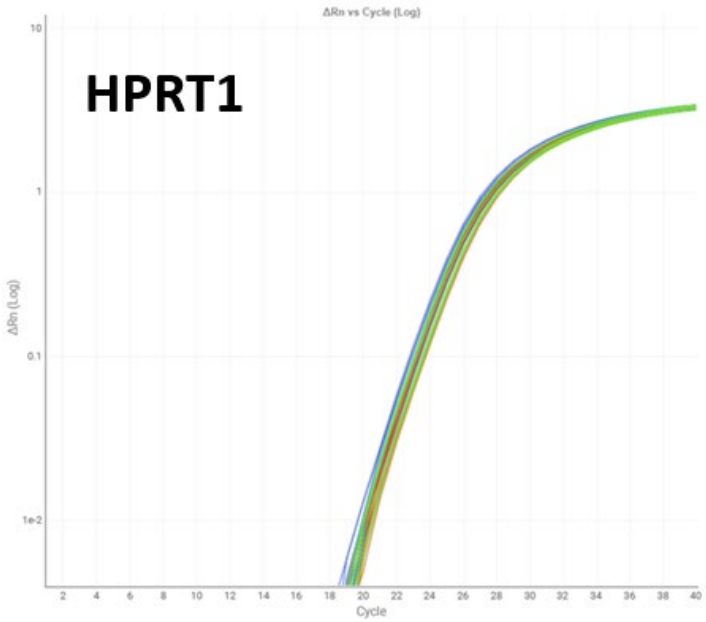
Biological Group	Sample ID	RNA Isolation		
		ng/ul RNA	260/280	260/230
Vehicle	C26-001	51.1	2.05	1.02
	C26-002	59.2	2.09	2.00
	C26-003	46.3	2.04	1.67
	C26-004	48.0	2.05	1.78
PG3 - 0.001%	C26-005	48.5	2.04	0.39
	C26-006	46.2	2.02	1.42
	C26-007	46.0	2.09	0.32
	C26-008	46.2	2.03	2.08
PG3-50 - 0.005%	C26-009	49.6	2.02	0.70
	C26-010	42.9	2.05	0.70
	C26-011	47.2	2.03	1.23
	C26-012	51.5	2.01	1.73

Endogenous Control Gene Selection

It is important to select an endogenous control gene that is consistently expressed in all of the samples of a comparison. Two candidate control genes (PPIA and HRPT1) were analyzed. The most consistent endogenous control gene was chosen based on the range scores given by the ThermoFisher Data

Connect RQ software. Lower range scores represent more consistent expression between samples in the study. Based on the range score, HRPT1 was selected as the endogenous control gene. Statistics (unpaired t-tests) were carried out for each comparison using dCq values normalized to HPRT1. qPCR amplification curves for HRPT1 are shown in Figure 3.

Figure 3. qPCR Amplification Curves for Selected Endogenous Control Gene HRPT1



Amplification curves are depicted in identifiable colors to represent each treatment group.

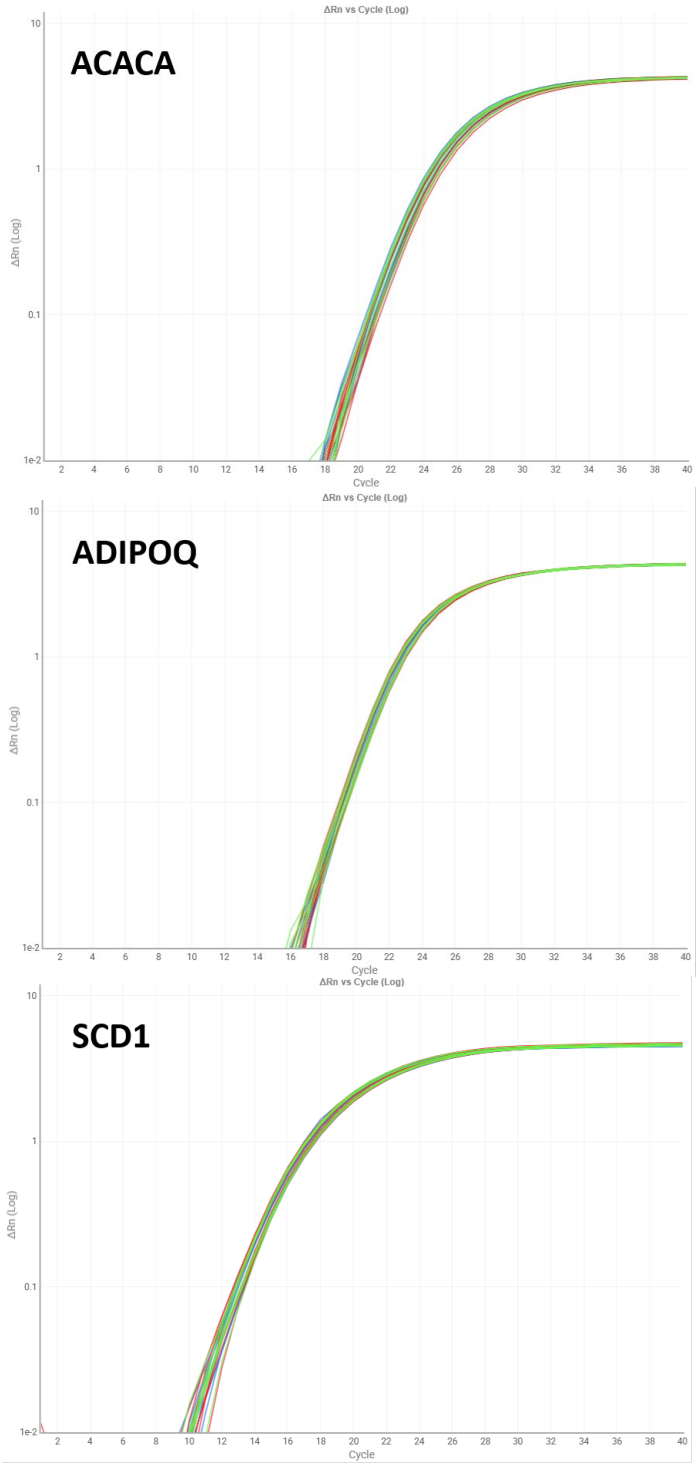
qPCR Data Quality and Statistical Data Analysis

qPCR data quality is assessed using a combination of factors, including visual analysis of the shape of the qPCR curve and the Cq value. Cq values are an indication of the total amount of transcript present in the sample and can impact the quality of the qPCR data. qPCR amplification takes place over a total of 40 cycles, and typically occurs before cycle 28. The relative amount of the gene transcript level is associated with the Cq value of the PCR reaction. Cq values typically correspond with the following:

- Cq values less than 28: associated with high transcript levels and robust, high quality qPCR data
- Cq values greater than 28: lower-level transcripts, less robust qPCR data; review data cautiously

All genes in the study amplified with Cq values $\leq$30, showing high quality qPCR curves (example shown below in Figure 4).

Figure 4. Example qPCR Amplification Curves for ACACA, ADIPOQ, and SCD1



Amplification curves are depicted in identifiable colors to represent each treatment group.