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AdipoLyze™ Lipolysis Detection Kit

Instructions for Use

Receiving Instructions

Protect from light, store kit at -20ºC

Safety Statements

THESE PRODUCTS ARE FOR RESEARCH USE ONLY. Not approved for human or veterinary use, for application to humans or animals, or for *in vitro* diagnostic or clinical procedures.

Assay Components

Assay contains sufficient materials for assessment of 1 x 96-well plate (~100 tests) and can be scaled to other multi-well plate formats including 384-well plates. White or black walled plates should be used (not supplied). Assay contents include:

1) 1 lyophilized vial of Assay Enzymes
2) 8 ml Assay Buffer
3) 320 µl Detection Reagent
4) 100 µl Glycerol Standard

Introduction

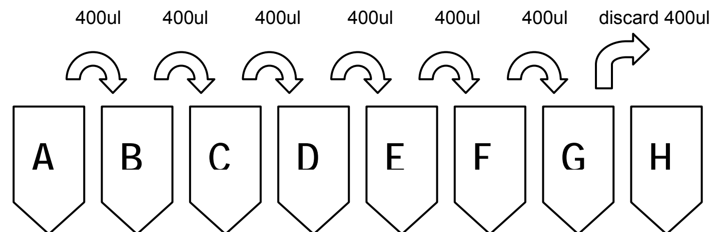
Preadipocytes and cell lines with adipogenic characteristics (ex: 3T3L1) are induced to differentiate and undergo adipogenesis, the process of lipid accumulation. Following adipogenesis, adipocytes can be induced to undergo lipolysis, which is the process by which cells break down triglycerides (lipid) into free fatty acids and glycerol for release into the blood stream (*in vivo*) or into the supernatant (*in vitro*). The AdipoLyze™ Lipolysis Detection kit is a fluorescent kit designed to quantify the glycerol released by cells undergoing lipolysis.

Glycerol Standard Curve Set up

Lonza recommends that you load standards in triplicate for best statistical results of averaged standard curves. Assay is optimized for detecting glycerol in a dynamic range of 108.6 µM – 0.4 µM (10 µg/ml – 0.04 µg/ml). It is not recommended to extrapolate "sample" concentrations using the derived equation from the curve if a sample's RFU values fall outside the fluorescent values provided by the curve.

1) Label eight 1.5 ml tubes (not provided) with the following concentrations and arrange in order in tube rack: 108.6 µM, 43.4 µM, 17.4 µM, 7.0 µM, 2.8 µM, 1.1 µM, 0.4 µM, and 0 µM.
2) Pipette 960 µl H_2O into the tube labeled "108.6 µM".
3) Pipette 600 µl H_2O into all remaining tubes.
4) Add 40 µl of the Glycerol Standard Solution to the tube labeled "108.6 µM". Close cap and vortex for 15 ± 5 seconds.
5) Prepare serial dilution by removing 400 µl from the tube labeled "108.6 µM" and adding the full 400 µl to the tube labeled "43.4 µM". Close cap and vortex for 15 ± 5 seconds.
6) Prepare next serial dilution by removing 400 µl from the tube labeled "43.4 µM" and adding the full 400 µl to the tube labeled "17.4 µM". Close cap and vortex for 15 ± 5 seconds.
7) Repeat above process with remaining tubes until you reach the tube labeled "0 µM". This tube should have 600 µl of sterile H_2O only. The 400 µl removed from the tube labeled "0.4 µM" should be discarded. See Figure 1 below.

Figure 1. Glycerol standard curve serial dilution diagram.



8) Load 50 µl of each standard into the corresponding wells on the 96-well plate (see Figure 2 – example plate layout - below).

Figure 2. General plate layout for alignment on plate reader.

	1	2	3	4	⟶	12
A	108.6 µM	108.6 µM	108.6 µM	sample	sample	Sample
B	43.4 µM	43.4 µM	43.4 µM	sample	sample	Sample
C	17.4 µM	17.4 µM	17.4 µM	sample	sample	sample
D	7.0 µM	7.0 µM	7.0 µM	sample	sample	sample
E	2.8 µM	2.8 µM	2.8 µM	sample	sample	sample
F	1.1 µM	1.1 µM	1.1 µM	sample	sample	sample
G	0.4 µM	0.4 µM	0.4 µM	sample	sample	sample
H	0.0µM	0.0µM	0.0µM	sample	sample	sample

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Sample Dilution and Set-up

Due to various levels of glycerol in the cell supernatant we recommend diluting your cell supernatants in water so that the glycerol levels fall within the standard curve readings. An example of an acceptable dilution would be 1:20, 1:50, or 1:100.

9) Using diluted cell supernatants, pipette 50 µl of unknown samples, in triplicate, into wells on the 96-well plate.

Fluorescent Plate Reader Set-up

10) Confirm plate reader settings are as follows:
    a. 570 nm excitation wavelength/595 nm emission wavelength with 590 cut off (if applicable)
    b. Room temperature, 6 reads per well (if available)
    c. Plate should be mixed 10 seconds before reading (if available).

Enzyme Rehydration

11) Remove crimped metal closure and rubber stopper from Assay Enzymes since the contents are under vacuum. BE VERY CAREFUL NOT TO LOSE ANY LYOPHILIZED MATERIAL.
12) Pipette 6 ml of Assay Buffer into the Assay Enzymes vial and mix gently (DO NOT VORTEX).
13) Add 150 µl of Detection Reagent to reconstituted Assay Enzymes to make "Enzyme/Detection Solution". Mix gently (DO NOT VORTEX).
14) Transfer approximately 6 ml of the solution to a 100 ml reagent reservoir (not included).
15) Using a multichannel pipette, transfer 50 µl "Enzyme/Detection Solution" to each well of 8 standards and any "Sample" wells. (WORK QUICKLY BUT CAREFULLY – reagents are light sensitive).
16) Mix gently at room temperature by using a plate shaker for 30±5 sec, or by pipetting, but do not introduce air bubbles into the wells. (If air bubbles occur, pop bubbles using a clean pipette tip for each well).
17) Incubate at room temperature for 60 - 120 minutes, protect from light. Reaction RFU values will peak between 60 and 120 minutes. Multiple time points can be collected if desired, but data should be analyzed with one consistent time point.
18) Mix plate again immediately before reading. (If plate reader does not have the mixing capability, gently mix the reagents with samples by using a plate shaker for 10±5 sec, or by pipetting).
19) Place plate on fluorescent plate reader press read/go. (Note plate orientation for correct alignment).

Data Analysis – Generate the standard curve

20) Average the RFU values from the standard curve and sample replicates.
21) Subtract "BLANK" (zero standard) averaged RFU values from each standard and sample.
22) Plot glycerol standard concentrations against corrected RFUs where x= concentration of glycerol standards and y= corrected RFUs (see Figure 3 as an example).

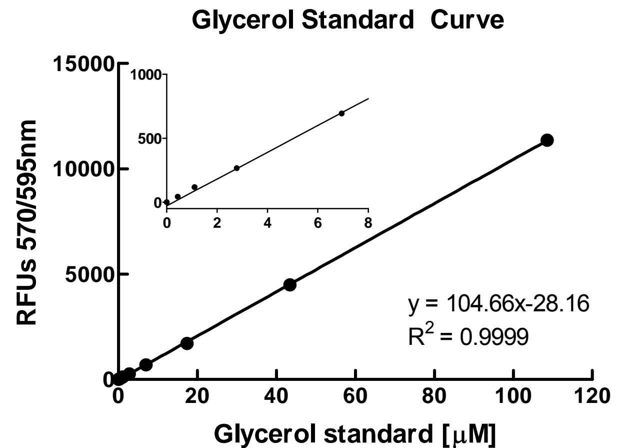

Figure 3. Typical linear glycerol standard curve. The sensitivity for glycerol detection is 0.44 µM (inset shows glycerol standard curve between 0-8 µM) with linear range up to 108.6 µM. [DO NOT USE THIS STANDARD CURVE TO ANALYZE YOUR DATA. THIS IS AN EXAMPLE.]

23) Calculate concentration of glycerol present in the sample by using the following equation:

    $y=mx+b$ where m=slope of the line; b=y-intercept and y=corrected RFUs for each sample

    $x=(y-b)/m$

Ordering Information

00193339	AdipoLyze™ Lipolysis Detection Kit, 1 x 96 wells	1 Kit
Related Products		
PT-5020	HPrAD-sq, Subcutaneous Preadipocytes	≥1,000,000 cells
PT-5001	HPrAD-sq, Subcutaneous Preadipocytes	≥4,000,000 cells
PT-5005	Visceral Preadipocytes	≥1,000,000 cells
PT-8002	PGM™-2 Preadipocyte Growth Medium-2 BulletKit™ [includes basal medium and SingleQuots™]	Kit
PT-7009	AdipoRed™ Assay Reagent	5 x 4 ml

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