



Triglyceride Assay Kit

100 point assay kit

Cat# TG-1-NC

INSTRUCTION MANUAL ZBM0013.01

STORAGE CONDITIONS

- **Reagents A & B, Buffers:**
 - Store at 2 - 8°C. See kit label for expiration date.

- **Glycerol Standard**
 - -20°C

For Research Use Only
Not For Use In Diagnostic Procedures

LIMITED PRODUCT WARRANTY

This warranty limits our liability to replacement of this product. No other warranties of any kind, expressed or implied, including without limitation, implied warranties of merchantability or fitness for a particular purpose, are provided by Zen-Bio, Inc. Zen-Bio, Inc. shall have no liability for any direct, indirect, consequential, or incidental damages arising out of the use, the results of use, or the inability to use this product.

ORDERING INFORMATION AND TECHNICAL SERVICES

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The contents of this kit are sufficient for the assay of 100 assay points in a 96 well plate format. The only reagents sold separately are Glycerol Reagent A (cat# RGTA-10) and the glycerol standard for the Triglyceride Assay kit (cat# TG-GLYSTAN). For additional reagents or formats, please order our bulk 500 point assay kit (cat# TG-5RB).

ITEMS INCLUDED IN THE KIT

ITEM	DESCRIPTION	UNIT	QTY	STORAGE
Wash buffer		BOTTLE	50ml	4°C
Lysis buffer		BOTTLE	25ml	4°C
Reagent A	Reconstitute w/ 11.0 ml deionized water prior to use	BOTTLE	11ml	4°C
Reagent B	Reconstitute w/ 2.5 ml deionized water prior to use	BOTTLE	2.5 ml	4°C
TG Glycerol standard	Glycerol @ 1mM [Reconstitute with 200 µl Standards Diluent to make the 200 µM glycerol standard; see page 4 for recommended dilution scheme]	VIAL	50 µl	-20°C
Standards Diluent		BOTTLE	2 ml	4°C
Tray	Clear polyvinyl tray for multi-channel pipetters	EACH	3	-----
Assay Plates, blank	96-well assay plate, blank	PLATE	2	-----

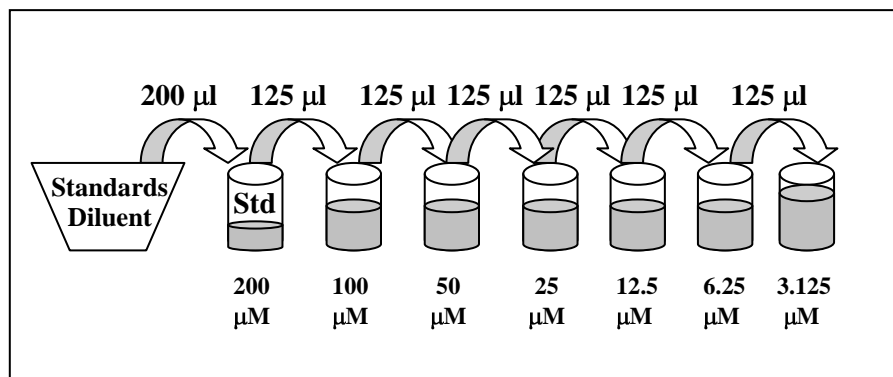
Other equipment/reagents required but not provided with the kit:

- Single-channel pipetter
- Multi-channel pipetter
- Plate reader with a filter of 540 nm
- Tubes to dilute glycerol standards
- Large gauge needle

ASSAY PROCEDURE

1. Warm the Wash buffer and Lysis buffer in a 37°C water bath.
2. Prepare the Reagent B by adding 2.5ml deionized water per bottle and gently invert. DO NOT VORTEX! Use a pipet to ensure that the powder is completely dissolved. Keep at room temperature. Store in a light protected bottle. Reconstituted Reagent B is stable for 60 days refrigerated (2-8°C); store any remaining solution refrigerated (2-8°C). Bring Reagent B to room temperature.
3. Remove all media from mature human adipocytes. Using about 15 ml of the wash buffer, wash the cells one time with 150 µl wash buffer. Label the disposable tray “wash buffer” and retain for later use.
4. Remove all Wash buffer. Using a new tray, add 15 µl Lysis buffer to each well. Incubate at 37°C - 50°C for 20 minutes.
5. After the incubation is complete, visually confirm cell lysis by checking the wells under a microscope. If cells are not fully lysed, incubate another 10 minutes.
6. Add 135 µl warm Wash Buffer to each well.
7. Add 20 µl Reagent B to each well. It is not necessary to mix at this time, however, gently tap the plate to help mix reagents. Incubate the plate at 37°C for 2 hours.
8. One hour prior to the assay, bring the glycerol standard and Reagent A to room temperature. Warm the Standards Diluent to 37°C. Prepare the standard curve as follows:

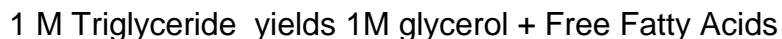
Pipette 200 µl of the Standards Diluent into the 1 mM glycerol standard tube provided and mix well by vortexing. This produces a diluted stock glycerol standard of 200 µM. Pipette 125 µl of diluent into 6 tubes (not provided). Using the newly diluted stock glycerol solution, prepare a dilution series as depicted below. Mix each new dilution thoroughly before proceeding to the next. The 200 µM stock dilution serves as the highest standard, and the diluent serves as the zero standard.



9. Also at this time prepare the Reagent A by adding 11.0 ml deionized water per bottle and gently invert. **DO NOT VORTEX!** Use a pipet to ensure that the powder is completely dissolved. Keep at room temperature. If using a Reagent A solution previously prepared and stored at 2-8°C, also bring to room temperature. Make sure there is enough Reagent A from one solution to treat all the points in the assay. It may be necessary to combine solutions. Store in a light protected bottle. Reconstituted Glycerol Reagent A is stable for 60 days refrigerated (2-8°C); store any remaining solution refrigerated (2-8°C).
10. To a blank 96 well plate, add 80 µl wash buffer to each well needed for the assay (NOTE: do not add Wash Buffer to the wells used for the standard curve).
11. Working with one row or column at a time, mix the lysates very well using a multi-channel pipet. Immediately transfer 20 µl per well of the lysates to the corresponding well of the plate containing the wash buffer. **This results in a Dilution Factor of 5.**
12. Prepare the standard curve. Pipet 100 µl of each standard into a well. (NOTE: Eight wells are necessary for the curve. If there are remaining wells on the assay plate, you can utilize the remaining wells. If not, a second plate is included in this kit).
13. Using the third tray, add 100 µl Reagent A to samples and standards. Mix by pipetting up and down one time. Incubate at room temperature for 15 minutes.
14. Read at 540 nm using a microtiter plate reader.

GLYCEROL STANDARD CURVE

This kit is designed to show relative lipid accumulation of experimental treatments compared to controls. The assay is based on the equation



The reagent measures the concentration of glycerol released after lysing the cells and hydrolyzing the triglyceride molecules. The triglyceride concentration can then be determined from the glycerol values.

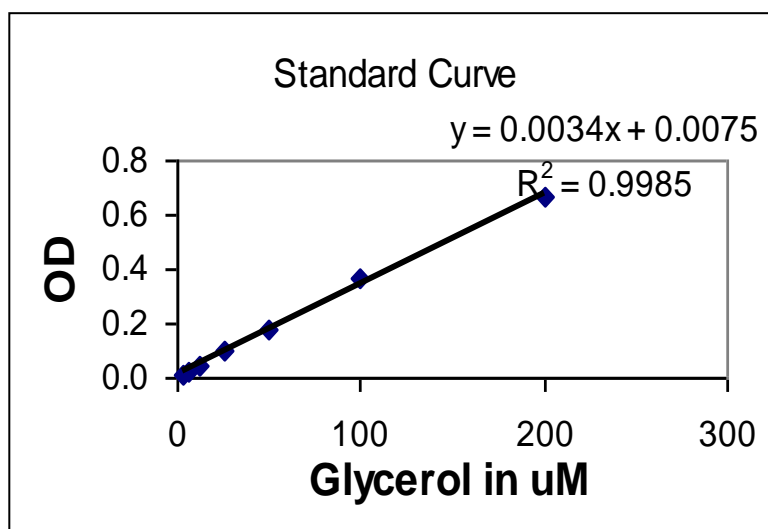
Generate standard curve: see example below

[DO NOT use this standard curve to generate your data. This is an example.]

Subtract the OD value of the 0 μ M standard from all OD values including the standard curve.

μ M Glycerol	OD	OD - blank
3.125	0.054	0.014
6.25	0.066	0.026
12.5	0.082	0.042
25	0.138	0.098
50	0.214	0.174
100	0.402	0.362
200	0.711	0.671

slope =	0.0034
intercept=	0.0075
r ² =	0.9985



y = observed O.D. minus the blank

x = concentration of glycerol in μ M

To calculate x for each y, (i.e. to change the observed O.D. into glycerol concentration) use the following equation:

$$y = (\text{slope}) \text{ times } (x) \text{ plus intercept}$$

$$y = mx + b \text{ so } x = (y - b) / m$$

$x = (y - 0.0075) / 0.003$ where 0.003 = slope of the line and 0.0075 = y intercept. Be careful to enter the proper sign for the y intercept value as it may be a negative number.

Any OD values greater than the highest standard (200 μ M) should be suspect. The compound should be re-assayed using a lower dose of the compound at treatment OR a dilute solution of the condition medium at the time of the assay.

The R² value should be equal or greater than 0.98 for the standard curve to be valid. Any R² values below 0.98, must have the standard curve run again.

Solve for the Total Glycerol concentration (i.e. total triglyceride concentration) for each OD. **Remember to include the Dilution Factor in the equation.**

Data is expressed as μM Glycerol.

NOTE: Any OD values that are negative after the blank is subtracted should be considered to be 0 for the OD value.

TROUBLESHOOTING

Problem	Suggestions
High background or the triglyceride reagent turns a darker color before the assay begins.	<ul style="list-style-type: none">• Use clean tray and tips• Change pipet tips frequently
Edge effects	<ul style="list-style-type: none">• Ensure a saturated humidity in the incubator to prevent evaporation from the outside wells
Inconsistent OD reading	<ul style="list-style-type: none">• Be careful when pipetting to avoid bubbles. If bubbles persist, burst the bubbles using a large gauge needle prior to reading and read the plate again.• Mix the lysates well before transferring the 20μl to the Wash buffer plate.

REFERENCES

1. Green, H. and Kehinde, O. (1974) Sublines of mouse 3T3 cells that accumulate lipid. *Cell* 1, 113-116.
2. Hauner, H., et al., (1989) *J. Clin. Invest.*(84), 1663-1670.
3. Kuri-Harcuch W, Wise LS, Green H. (1978) Interruption of the adipose conversion of 3T3 cells by biotin deficiency: differentiation without triglyceride accumulation. *Cell* 14:53-58.

APPENDIX A: PLATE LAYOUT

H	G	F	E	D	C	B	A	
								1
								2
								3
								4
								5
								6
								7
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								9
								10
								11
								12

APPENDIX B: PROCEDURE FLOWCHART

