# Serum Triglyceride Assay Kit

Cat# STG-1-NC

**INSTRUCTION MANUAL ZBM0036.06**

## STORAGE CONDITIONS

- **Diluent & Buffers**
  - **Room Temperature**
- **Glycerol Reagent A:**
  - **+4°C Use reconstituted Glycerol Reagent A within 7 days.**
- **Glycerol Standard & Reagent B**
  - **-20°C NEW REAGENT B STORAGE TEMPERATURE**

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# INTRODUCTION

This kit is designed to accurately determine the amount of triglyceride present in blood serum or plasma of humans, mice, rats, and other animals in a 96-well format for increased throughput analysis. Blood can be collected in plain evacuated tubes or in the presence of common anti-coagulants: heparin, EDTA, sodium citrate, or ammonium oxalate. Serum should be separated from clotted blood by centrifugation as soon as possible and may be stored frozen (-20°C) prior to analysis.

## PRINCIPLE OF THE ASSAY

Enzymatic hydrolysis of serum triglycerides by lipase generates free fatty acids and glycerol. The glycerol released is phosphorylated by adenosine triphosphate (ATP) forming glycerol-1-phosphate (G-1-P) and adenosine-5'-diphosphate (ADP) in the reaction catalyzed by glycerol kinase. G-1-P is then oxidized by glycerol phosphate oxidase to dihydroxyacetone phosphate (DAP) and hydrogen peroxide ($H_2O_2$). A quinoeimine dye is produced by the peroxidase catalyzed coupling of 4-aminoantipyrine (4-AAP) and sodium N-ethyl-N-(3-sulfopropyl)m-anisidine (ESPA) with $H_2O_2$, which shows an absorbance maximum at 540nm. The increase in absorbance at 540nm is directly proportional to glycerol (and triglyceride) concentration of the sample.

**Triglyceride ⟶ Fatty Acids + Glycerol**

**GLYCEROL + ATP ⟶ G-1-P + ADP**

**G-1-P + $O_2$ ⟶ DAP + $H_2O_2$**

**$H_2O_2$ + 4-AAP + ESPA ⟶ Quinoneimine dye + $H_2O$**

# ITEMS INCLUDED IN THE KIT

The contents of this kit are sufficient for the assay of 1, 96 well plate. For additional reagents or formats, please order our bulk kit (cat# STG-5RB).

| ITEM | DESCRIPTION | UNIT | QTY | STORAGE |
|---|---|---|---|---|
| Reagent A | Reconstitute w/ 11.0 ml deionized water prior to use. Use reconstituted reagent within 7 days! | BOTTLE | 1 | 4°C |
| Reagent B | 2.5 ml Reagent B solution new storage temperature | BOTTLE | 1 | -20°C |
| Glycerol standard | 10 µl Glycerol @ 10mM [Reconstitute with 40 µl Standards Diluent to make the 2 mM glycerol standard; see page 4 for recommended dilution scheme] | VIAL | 1 | -20°C |
| Diluent | Standards Diluent, 2.0ml | BOTTLE | 1 | 4°C |
| Tray | Clear polyvinyl tray for use with multi-channel pipetters | EACH | 2 | ROOM TEMP. |
| Assay Plates, blank | 96-well assay plate, blank | PLATE | 2 | ROOM TEMP. |

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

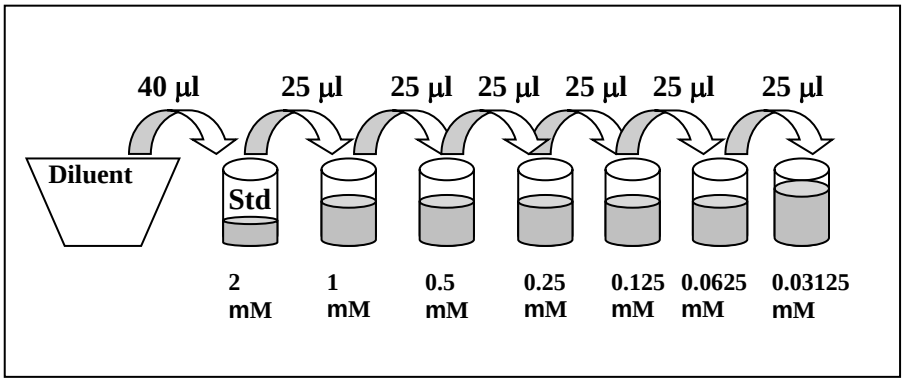
- Single-channel pipetter
- Multi-channel pipetter
- Plate reader with a filter of 540 nm
- Tubes for diluting glycerol standards

# REAGENT PREPARATION

1. Thaw Reagent B to room temperature and gently invert. DO NOT VORTEX! Reagent B may be refrozen (-20°C) and stored. Avoid repeated freeze/thaws.

2. Prepare the Glycerol Reagent A by adding 11.0 ml deionized water per bottle and gently invert. DO NOT VORTEX! Use a pipet to insure 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. Store in a light protected bottle. Reconstituted Glycerol Reagent A is stable for 7 days refrigerated (2-8°C); store any remaining solution refrigerated (2-8°C).

3. Prepare Glycerol Standards using the 10mM glycerol standard provided. Briefly centrifuge thawed 10mM glycerol standard to collect all liquid in the bottom of the tube. Add 40 µl of diluent to 10mM stock glycerol standard to generate a 2mM glycerol standard. Add 25 µl of diluent to 6 tubes (not provided). Prepare the dilution series as outlined below. Mix each new dilution thoroughly before proceeding to the next. The 2 mM solution serves as the highest standard, and the diluent serves as the zero standard.




# ASSAY PROCEDURE

## STEPWISE GLYCEROL AND TRIGLYCERIDE MEASUREMENT

1. Warm reconstituted Glycerol Reagent A and Reagent B to room temperature.

2. Add 80 µl of reconstituted Glycerol Reagent A to the appropriate number of wells for samples and standard curve in the provided 96-well plate.

3. Add 5.0 µl of prepared standard curve dilutions to wells containing Glycerol Reagent A. Transfer 1.0 - 5.0 µl of serum or plasma samples to remaining wells. Incubate at room temperature for 15 minutes

4. Read at 540 nm using a microtiter plate reader and record absorbance values as Glycerol Readings. (Glycerol standards from this reading are used to calculate total glycerol)

5. Add 20 µl of Reagent B to each well and incubate and additional 15 minutes at room temperature. Record absorbance values as Total Triglyceride Readings. (Glycerol standards from this reading are used to calculate combined glycerol and triglyceride levels.)

# DATA ANALYSIS

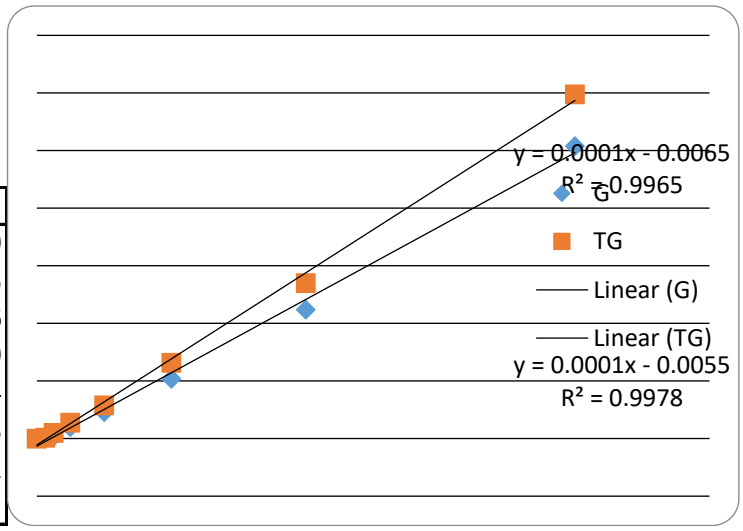
## STEPWISE GLYCEROL AND TRIGLYCERIDE MEASUREMENT

Generate standard curves: see example below

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

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

| uM Glycerol | Gly Std OD | TG std OD | Gly - Blank | TG -Blank |
|---|---|---|---|---|
| 2000 | 0.293 | 0.342 | 0.254 | 0.299 |
| 1000 | 0.151 | 0.178 | 0.112 | 0.135 |
| 500 | 0.091 | 0.109 | 0.052 | 0.066 |
| 250 | 0.062 | 0.072 | 0.023 | 0.029 |
| 125 | 0.049 | 0.057 | 0.01 | 0.014 |
| 62.5 | 0.041 | 0.048 | 0.002 | 0.005 |
| 31.25 | 0.039 | 0.044 | 0 | 0.001 |
| 0 | 0.039 | 0.043 | | |




| | Gly | TG |
|---|---|---|
| slope = | 0.0001 | 0.0001 |
| intercept= | 0.0065 | 0.0055 |
| $r^2$= | 0.9965 | 0.9978 |

**y = observed O.D. minus the blank**

x = concentration of glycerol in μM

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

y=(slope) times (x) plus intercept

y=mx+b so x=(y-b)/m

x=(y – 0.0065)/0.0001 where 0.0001= slope of the line and 0.0065= 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 (2000 μM) 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 $R^2$ value should be equal or greater than 0.98 for the standard curve to be valid. Any $R^2$ 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 any 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.

1. Calculate the values for glycerol present in sample by using the initial glycerol standards reading.
2. Calculate the values for the combined glycerol and triglyceride by using the second glycerol standards reading.
3. Subtract the glycerol levels from step 1 from the combined glycerol and triglyceride values in step 2. This gives the value of total triglyceride in the sample.

## TROUBLESHOOTING GUIDE

| Problem | Suggestions |
|---|---|
| High background or the triglyceride reagent turns a darker color before the assay begins. | • Use clean tray and tips<br>• Change pipet tips frequently |
| Edge effects | • Ensure a saturated humidity in the incubator to prevent evaporation from the outside wells |
| Inconsistent OD reading | • 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.<br>• 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 |
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# APPENDIX B: PROCEDURE FLOWCHART

## Stepwise Glycerol and Triglyceride Detection

Reconstitute Glycerol Reagent A and thaw Reagent B according to instructions

↓

Add 80µl/well reconstituted Glycerol Reagent A

↓

Add 5µl/well glycerol standards and 1-5µl/well samples.

↓

Incubate 15 minutes @ room temperature. Measure the optical density of each well at 540 nm using a spectrophotometer plate

↓

Add 20µl/well Reagent B

↓

Incubate 15 minutes @ room temperature. Measure the optical density of each well at 540 nm using a spectrophotometer plate reader.