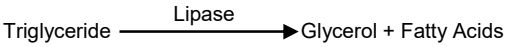
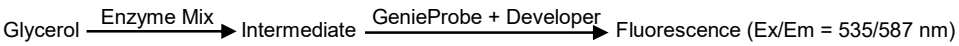
# Triglyceride (TG) Fluorometric Assay Kit (BN00838)

**(Catalog #BN00838; 100 assays; Store at -20°C)**

## I. Introduction:

Triglycerides (TG) are the main constituents of vegetable oil, animal fat, LDL and VLDL, and play an important role as transporters of fatty acids as well as an energy source. TG is broken down into fatty acids and glycerol, after which both can serve as substrates for energy producing and metabolic pathways. High blood levels of TG are implicated in atherosclerosis, heart disease and stroke as well as in pancreatitis. Assay Genie's Triglyceride Assay kit is suitable for measuring triglyceride levels in samples, which contain reducing substances that may interfere with oxidase-based assays. In this assay, TG is hydrolyzed to glycerol and fatty acid. The glycerol reacts with Triglyceride Enzyme Mix to form an intermediate product, which in turn reacts with GenieProbe & Developer to generate the fluorescence. The generated fluorescence is directly proportional to the amount of triglycerides. This high-throughput adaptable assay kit is simple, sensitive and easy to use & can detect less than 0.4 µM triglycerides in a variety of samples.




## II. Application:

- Measurement of triglycerides in various tissues, cells & other biological fluids
- Analysis of lipid metabolism
- Mechanistic study of cardiovascular disease

## III. Sample Type:

- Animal tissues: Liver, pancreas, heart etc.
- Cell culture: adipocytes etc.
- Biological fluids: serum, plasma & saliva.

## IV. Kit Contents:

| Components | BN00838 | Cap Code | Part Number |
|---|---|---|---|
| Triglyceride Assay Buffer | 25 ml | WM | BN00838-1 |
| GenieProbe (in DMSO) | 0.4 ml | Blue | BN00838-2 |
| Lipase (Lyophilized) | 1 vial | Orange | BN00838-3 |
| Triglyceride Enzyme Mix (Lyophilized) | 1 vial | Green | BN00838-4 |
| Triglyceride Developer (Lyophilized) | 1 vial | Red | BN00838-5 |
| Triglyceride Standard (1mM) | 0.3 ml | Yellow | BN00838-6 |

## V. User Supplied Reagents and Equipment:

- 96-well white plate with flat bottom
- Multi-well spectrophotometer (ELISA reader)

## VI. Storage and Handling:

Store kit at –20°C, protected from light. Warm Assay Buffer to room temperature before use. Briefly centrifuge small vials prior to opening. Read the entire protocol before starting the experiment.

## VII. Reagent Preparation and Storage Conditions:

- **Triglyceride (TG) Fluorometric Assay Kit:** Ready to use as supplied. Warm to room temperature before use. Store at -20°C.
- **Lipase and Triglyceride Enzyme Mix**: Reconstitute with 220 µl Triglyceride Assay Buffer. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Keep on ice while in use. Stable for 2 months at -20°C.
- **Triglyceride Developer**: Reconstitute with 220 µl $dH_2O$. Pipette up and down to dissolve completely. Aliquot and store at -20°C. Avoid repeated freeze/thaw. Stable for 2 months at -20°C.
- **Triglyceride Standard:** Storage at -20°C may cause the Triglyceride Standard to separate from the aqueous phase. To re-dissolve, tightly close the cap & put the vial in boiling water for 1 min (solution will turn cloudy). Vortex for 30 sec, the Standard solution will become clear. Repeat the heat and vortex step one more time. The Triglyceride Standard solution is now ready to be used.

## VIII. Triglyceride Assay Protocol:

1. **Sample Preparation:** Serum samples can be measured directly. Tissue (10 mg) or cells (1 x $10^6$) should be rapidly homogenized with 100 µl ice cold Triglyceride Assay Buffer for 10 min. on ice. Centrifuge at 12000 rpm for 5 min. Collect the supernatant. Saliva should be briefly spun down at 5000 rpm for 2 min. Collect the supernatant. Add 1-50 µl sample into a 96 well plate and adjust the volume to 50 µl/well with Triglyceride Assay Buffer.

   **Notes:**

   **a.** For unknown samples, we suggest testing several doses to ensure the readings are within the Standard Curve range.

   **b.** NADH in samples will generate background. For samples having high NADH levels, prepare parallel sample well(s) as background control.

2. **Standard Curve Preparation:** Dilute Triglyceride Standard to 20 µM (20 pmol/µl) by adding 20 µl of 1 mM Triglyceride Standard to 980 µl $dH_2O$. Mix well. Add 0, 2, 4, 6, 8 & 10 µl of diluted 20 µM Triglyceride Standard into a series of wells in 96 well plate to generate 0, 40, 80, 120, 160 & 200 pmol/well Triglyceride Standard. Adjust volume to 50 µl/well with Triglyceride Assay Buffer.
3. **Lipase Treatment:** Add 2 µl of Lipase to Standard and sample wells. Mix and incubate at 37$^0$C for 20 minutes.
4. **Reaction Mix:** Mix enough reagents for the number of assays (samples and Standards) to be performed. For each well, prepare 50 µl Reaction Mix containing:

| | Reaction Mix | Background Control Mix | |
|---|---|---|---|
| Triglyceride Assay Buffer | 45 µl | 47 µl | |
| Triglyceride (TG) Fluorometric Assay Kit | | 1 µl | 1 µl |
| Triglyceride Enzyme Mix | 2 µl | ---- | |
| Triglyceride Developer | 2 µl | 2 µl | |

Add 50µl of the Reaction Mix to each well containing the Standard & test samples and 50µl of background control mix to sample background control well(s). Mix well.

5. **Measurement:** Incubate the reaction for 30 minutes at 37$^0$C, protected from light. Measure fluorescence at Ex/Em = 535/587 nm in a micro plate reader.
6. **Calculation:** Subtract 0 Standard reading from all readings. Plot the Triglyceride Standard curve. If sample background control reading is significant, subtract the background control reading from sample readings. Apply the corrected sample readings to the Triglyceride Standard Curve to get B pmol of Triglycerides in the sample wells.

**Sample Triglyceride concentration = B/V x Dilution Factor = pmol/ml = nM**

Where: **B** is the amount of Triglycerides from the Standard Curve (pmol).
**V** is the sample volume used in the reaction well (ml).

Triglycerides in samples can also be expressed in pmol/mg or mmol/dL of sample.

Triglyceride-triolein molecular weight: 885.43 g/mol.

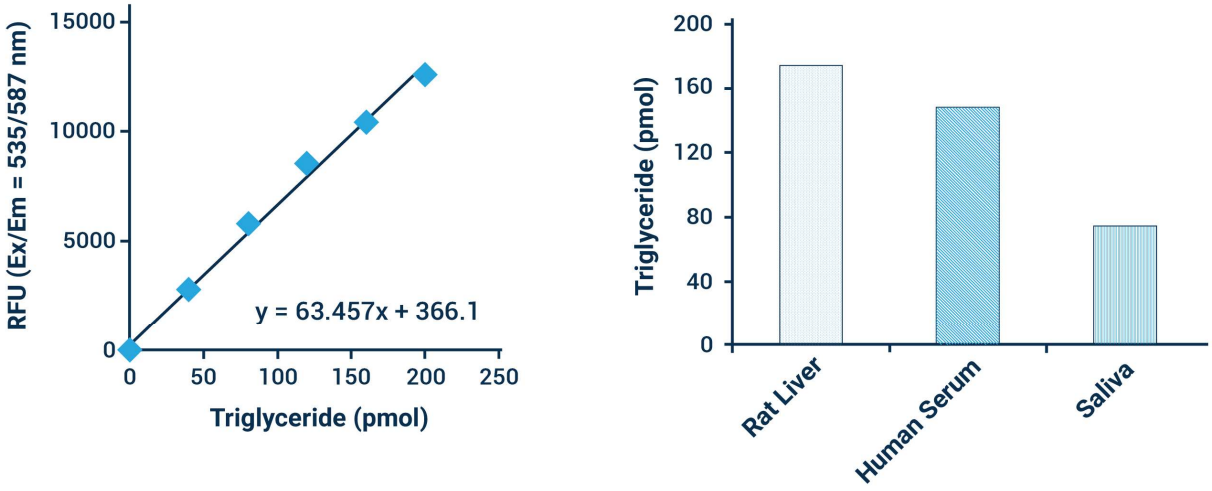



**Figure 1**. Triglyceride Standard Curve (a), measurement of triglyceride levels in rat liver (~3 µg) & human serum (2 µl of 1:10 diluted) & saliva (10 µl). Assays were performed following the kit protocol.

***FOR RESEARCH USE ONLY! Not to be used on humans.***