A convenient, easy-to-follow shortened protocol is now being provided with this assay.
For a detailed protocol go to www.caymanchem.com/pdfs/10010303.pdf

Triglyceride Colorimetric Assay Kit Short Protocol

Item No. 10010303

REAGENT PREPARATION

1. **Triglyceride Standard - (Item No. 10010509)** - The vial contains 400 µl of a 1,000 mg/dl solution of Triglyceride Standard. It is ready to use as supplied.

2. **Standard Diluent Assay Reagent (5X) - (Item No. 700732)** - Dilute the contents of the vial (12 mls) with 48 ml of HPLC-grade water; stable six months at room temperature.

3. **Sodium Phosphate Assay Buffer - (Item No. 700003)** - Dilute the contents with 16 ml of HPLC-grade water (final formulation is 50 mM sodium phosphate, pH 7.2); stable six months at room temperature.

4. **Triglyceride Enzyme Mixture - (Item No. 10010511)** - Reconstitute the contents with 1 ml of HPLC-grade water and transfer to a 15 ml centrifuge tube wrapped in aluminum foil. Add 14 ml of the diluted Assay Buffer and mix by inversion. *NOTE: A portion of the 14 ml should be used to rinse any residual solution from the vial. Stable 1 month at 4°C. Do NOT Freeze!* A slight pink discoloration may occur but will not affect assay performance.

STANDARD PREPARATION

Label 8 clean test tubes 1-8. Add 400 µl of diluted Standard Diluent to tube 1 and 200 µl to tubes 2-8. Add 100 µl of Triglyceride Standard to tube 1 and mix thoroughly (concentration = 200 mg/dl (2.26 mmol/L)). Serially dilute the standard by removing 200 µl from tube 1 and adding it to tube 2; mix thoroughly. Next, remove 200 µl from tube 2 and place it into tube 3; mix thoroughly. Repeat this process for tubes 4-7. Tube 8 only has diluted Standard Diluent and is used as the blank. The concentration of these standards will be: 200, 100, 50, 25, 12.5, 6.25, 3.13, and 0 mg/dL. Use standards within one hour.

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PERFORMING THE ASSAY

1. **Triglyceride Standard Wells** - Add 10 µl of standard (tubes 1-8) to duplicate wells.
2. **Sample wells** - Add 10 µl of sample to duplicate wells (triplicate recommended).
3. **Initiate** the reaction by adding 150 µl of diluted Enzyme Mixture to each well.
4. Carefully **shake** the microwell plate for a few seconds to mix. **Cover** with the plate cover.
5. **Incubate** the plate for 15 minutes at room temperature.
6. **Read** the absorbance at 530-550 nm.

CALCULATIONS

1. Calculate the average absorbance of each standard and sample.
2. Subtract the absorbance value of standard 8 (0 mg/dl) from itself and all other values (both standards and samples). This is the corrected absorbance.
3. Graph the corrected absorbance values of each standard as a function of the final triglyceride concentration (mg/dl) and fit to a linear regression.
4. Calculate the values of triglyceride samples using the equation below.

$$\text{Triglycerides (mg/dl)} = \left[\frac{\text{(Corrected absorbance) - (y-intercept)}}{\text{Slope}}\right]$$