

Triglyceride Assay; Microplate (Pointe Scientific)

Materials Required:

1. Pipettes: 3.5uL – 350uL
2. Microplate
3. Timer
4. Oven (37°C)
5. Microplate Shaker
6. Triglyceride GPO Reagent (Pointe Scientific Cat #T7532)
7. Triglyceride Standard (Pointe Scientific Cat #T7531)
8. Quality Control (Multi-analyte Cholestech #88773)
9. Tecan Microplate Reader with 500nm Absorbance Filter
10. Other documents:
 - a. Triglyceride Assay_Microplate Well Map
 - b. Triglyceride Assay_Microplate_Results Template
 - c. Tecan Spectrophotometer Instructions
 - d. Tecan Single Standard Video

Sample Collection/ Storage:

1. Clear, unhemolyzed serum is sample of choice.
2. Serum (gold-topped tube): Gently invert vial 8-10x immediately following collection, let sit 30min at RT then centrifuge at 1000g's (2.6-3.0 RPM) for 15min.
3. Following centrifugation, immediately pipette serum into appropriately labeled micro-centrifuge tubes.
4. Samples should be run immediately or frozen for batch analyses.

Procedure:

1. Place microplate shaker in oven and pre-heat oven to 37°C.
2. Create microplate well map using the 'Triglyceride Assay_Microplate Well Map' as a guide. Enter your blanks, standards, and samples in duplicate as indicated.
Note: You will need to follow the 'Triglyceride Assay_Microplate Well Map' precisely if you want to use the excel results template ('Triglyceride Assay_Microplate_Results Template') to analyze your results.
3. Add 350uL of triglyceride GPO reagent into all wells. Watch for bubbles; you may want to use reverse pipetting.
4. Place microplate into oven for 5 minutes to warm reagent.
5. Immediately and carefully, pipette 3.5uL of appropriate solution (standard, control, sample, nothing in the blank wells) using your microplate well map as a guide.

6. Place the microplate on the plate shaker (in oven), set rotation for 450-500 rpm, and incubate for 30 minutes. Read the plate immediately after incubation; do not let it cool to room temperature.
7. During incubation prepare the plate reader. Refer to the 'Tecan Spectrophotometer Instructions' and the 'Tecan Single Standard Video' if necessary.
8. To use the excel results template; copy and paste the first set of values from the Tecan results onto the first worksheet (titled 'Raw Data') of the 'Triglyceride Assay_Microplate_Results Template.' The data will automatically populate into the next worksheet (titled 'Results') and the results will automatically get calculated.

Notes: Final color is stable for 60 minutes.

Reportable Range (linearity) is 0 – 1000mg/dL. If a sample exceeds the upper linearity limit it needs to be diluted with isotonic saline and rerun. Multiply results by 2.

Verifying Results:

1. Make sure your controls are within range.
2. Verify that your coefficient of variation for your sample duplicates are within acceptable limits ($\leq 15\%$), rerun if necessary.