# PagLab Protocols

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## TG Analysis of Liver

1. Homogenize approximately 20 mg of liver in 1 ml PBS/10 mM EDTA pH 7.4.

2. Add 2 ml of isopropanol-hexane-water (IHW: 80:20:2, v/v/v) to glass tubes.
    a. 78.4 : 19.6 : 2.0 ml for 100 ml total volume.
    b. Glass tubes should be soaked overnight in dd $H_2O$ and dried.

3. Add 200 ul of homogenate to each glass tube from #2 above.
    a. Mix by vortexing.
    b. Incubate covered with aluminum foil for 30 minutes at room temperature.

4. Add 500 ul hexane-ether (1:1) to each tube.
    a. 5 : 5 ml for 10 ml total volume.
    b. Mix by vortexing.
    c. Incubate covered with aluminum foil for 10 minutes at room temperature.

5. Add 1 ml of dd $H_2O$ to separate phases.
    a. Mix by vortexing.
    b. Incubate covered with aluminum foil for 20 minutes at room temperature.

6. Remove 900 ul of organic phase and place into glass GC vials.
    a. Evaporate under $N_2$ gas.

7. Add 500 ul of Infinity TG reagent to each vial, including vials containing TG standard dilutions (0, 0.03125, 0.0625, 0.125, 0.25, 0.5, 1; 12.5 ul standard per 500ul TG reagent)
    a. Cap vials.
    b. Incubate at 37°C for 90 minutes with shaking.
    c. After incubation, dilute 5-fold.

8. Transfer 200 ul from vial to 96-well plate.

9. Determine $A_{540}$ against standard curve (TG standard solution = 200mg/dl).