Measurement of Liver Triglyceride Content

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[Abstract] This assay is designed to measure relative lipid accumulation of experimental treatments compared to controls. The reagent measures the concentration of glycerol released after lysing the cells and hydrolyzing the triglyceride molecules. The triglyceride concentration can then be determined from the glycerol values.

Materials and Reagents

1. EtOH
2. KOH
3. $MgCl_2$
4. Triglyceride (GPO Trinder) reagent A (Sigma-Aldrich, catalog number: 337-40A)
   [Please note that this product has been replaced by free glycerol reagent (F6428) from Sigma]
5. Glycerol Standards (Sigma-Aldrich, catalog number: 339-11 or G7793)
6. Ethanolic KOH (see Recipes)

Equipment

1. Microfuge tube
2. Glycerol
3. Microreader
4. Cuvette

Procedure

A. Creation of saponified, neutralized liver extract
   1. Moved 100-300 mg of liver to pre-weighed microfuge tube.
   2. Record tube + liver weight.
   3. Add 350 µl ethanolic KOH.

4. Incubate overnight 55 °C
5. Vortex early in incubation till the tissue is completely digested.
6. By morning, tissue should be digested and no oil layer should be visible (if oil layer is present then one must digest longer, and may need more ethanolic KOH).
7. Bring volume to 1,000 µl with H_2O: EtOH (1:1).
8. Spin in microfuge 5 min, move supernatant to new tube.
9. Bring volume of supernatant to 1,200 λ with H_2O: EtOH (1:1), vortex.
10. Move 200 µl to new eppendorf tube, add 215 µl 1 M $MgCl_2$, vortex.
11. 10 min on ice.
12. Microfuge 5 min, move supernatant to new tube.

B. Assay of glycerol content

1. Reconstitute reagent A according to instructions for determination of glycerol content.
2. Pipette 1 ml of reconstituted reagent A into cuvettes.
3. Add samples, standards and blank.
   Standards: 10 µl + 20 µl H_2O
   Samples: 30 µl
   Blank: 30 µl H_2O
4. Shake.
5. Incubate 15 min & read at 540 nm.

C. Calculation of liver TG content

1. Note that the Sigma glycerol standards are expressed as triglyceride (triolein) equivalents.
2. Subtract blank from all samples/standards.
3. Create standard curve by linear regression.
4. Determine cuvette (triolein equiv) (CTE) glycerol concentration by comparison to standard curve.
5. Liver TG content (in mg TG / gram liver)
   = CTE (mg/dl)*(10/30)*(415/200)*0.012 (dl)/wt (gr)

Recipes

1. Ethanolic KOH (2 parts EtOH: 1 part 30% KOH)
2. 1 M $MgCl_2$
3. H_2O: EtOH (11)

Acknowledgments

This protocol was previously used in Norris *et al.* (2003).

References

1. Norris, A. W., Chen, L., Fisher, S. J., Szanto, I., Ristow, M., Jozsi, A. C., Hirshman, M. F., Rosen, E. D., Goodyear, L. J., Gonzalez, F. J., Spiegelman, B. M. and Kahn, C. R. (2003). Muscle-specific PPARgamma-deficient mice develop increased adiposity and insulin resistance but respond to thiazolidinediones. *J Clin Invest* 112(4): 608-618.

