149. Determining Eukaryotic Microalgal TAG Yield Using a Commercial Serum Triglyceride Determination Kit

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Project Goals: In response to stress, eukaryotic microalgae produce triacylglycerides (TAGs) that are stored in lipid bodies; the TAG is an excellent substrate for biodiesel production. Assays that have been developed for assessing microalgal TAG content are time-consuming and require equipment and technical expertise that is often not available to groups interested in pursuing the development of this technology. We have optimized a rapid and quantitative colorimetric TAG assay for two microalgae, the green *Chlamydomonas reinhardtii* and the red *Cyanidioschyzon merolae*, using an inexpensive and commercially available kit developed to monitor serum TAG levels in human blood samples.

TAG quantitation in *C. reinhardtii* commonly utilizes a version of the procedure developed in the Benning lab (described in detail in 1): cells are pelleted and extracted with organic solvents; the extract is run on thin-layer chromatography plates; the TAG band is scraped off and converted to fatty-acid methyl esters (FAMEs); and the FAMEs are quantitated using mass-spec (GC-MS) or flame ionization detection (GC-FID) gas chromatography, with peaks then integrated to calculate total TAG yield. An alternative approach, developed in the Hildebrand lab for diatoms (2,3), quantitates the signal from BODIPY 493/503 (Molecular Probes), which fluoresces in a neutral-lipid environment (but see refs xy), using an imaging flow cytometer. The first method has been described as "tedious and time consuming" (4); the second requires determination that, for a given microalgal strain, only TAG is BODIPY-positive; and both require sophisticated equipment operated by experienced users. Research labs without access to such resources usually report their results using versions of Bligh and Dyer assays (5) that measure both polar and neutral lipids and hence give no information about TAG content.

Commercially available kits allow the performance of a colorimetric assay, using a standard bench-top spectrophotometer, that measures the glycerol released after TAGs are digested with TAG lipases. We previously (6) quantitated the TAG content of purified lipid bodies from *C. reinhardtii* using such a kit, and obtained values very similar to those obtained using GC-MS. We report here the use of such a kit to evaluate the TAG content of whole cells. In optimizing the assay, we used *C. reinhardtii* and *C. merolae*, which have very different pigment profiles, and heeded the potential artifacts in the assay described in a paper on *Drosophila* (7).

Take it from here!

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