# Bioluminescent Assays For Measuring Steatosis and Insulin Action

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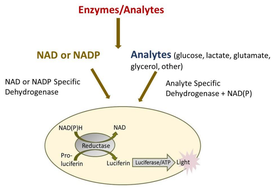
**Poster #3035**

## 1. Bioluminescent Metabolite Detection Assays

Nonalcoholic fatty liver disease (NAFLD), and its more serious form nonalcoholic steatohepatitis (NASH), are conditions in which lipids accumulate in the liver. To study these diseases, it is not only important to find good cellular models of steatosis, but it is also important to develop better assays to measure changes in lipid accumulation. Staining is routinely used to monitor lipid levels, but this is often nonspecific, somewhat laborious, and not quantitative. There are quantitative assays available, but most require an organic extraction, which is also undesirable.




We have developed a core bioluminescent technology that couples specific metabolite dehydrogenases to the production of NAD(P)H and the generation of light. These assays rely on detergents for lysis and extraction of lipids, hence, by utilizing specific dehydrogenases, lipases, and esterases, we can quantify triglyceride, cholesterol, and cholesterol esters without the requirement for an organic solvent.

## 2. Metabolite Detection Technology: light ≈ [metabolite]

**Metabolites**
- Glutamine
- Triglycerides
- Cholesterol Esters
- Aspartate
- Other

→ **Enzymatic Conversion** →

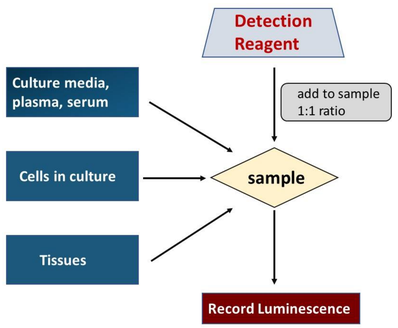
**Metabolites for Dehydrogenases**
- Glucose
- Lactate
- Glutamate
- Glycerol
- Cholesterol
- Other

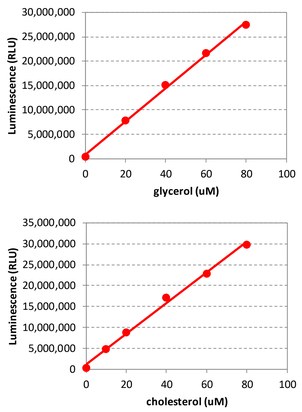
→ **Detection Reagent** → Light

**Detection Reagent** →
- Specific Dehydrogenase
- NAD/NADP
- Reductase
- Reductase Substrate
- Utra-Glo Luciferase/ATP

*NAD(P)H Detection*

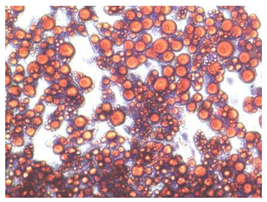
## 3. General Protocol and Standard Curves

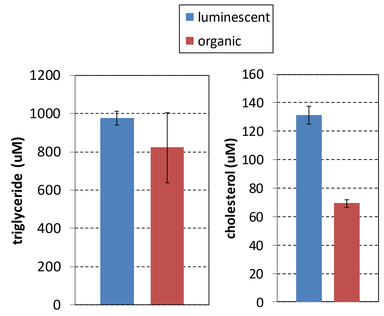



## 4. Organic vs Detergent Extraction of Lipids

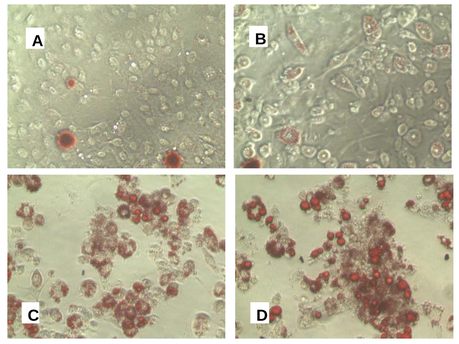
Lipids were extracted from mature adipocytes by organic solvent or detergent lysis solutions and assayed with the bioluminescent assays.



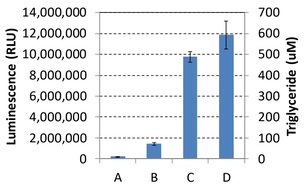



## 5. Lipid Accumulation During Adipocyte Differentiation

Oil Red O Staining

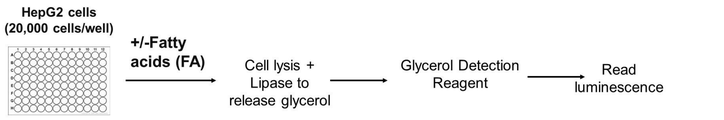



Triglyceride Detection Assay

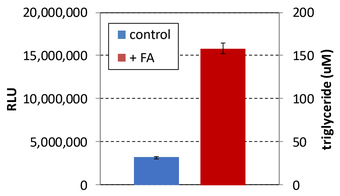



A – fibroblasts
B – differentiation stage 1
C – differentiation stage 2
D – mature adipocytes

## 6. HepG2 as a model for NAFLD

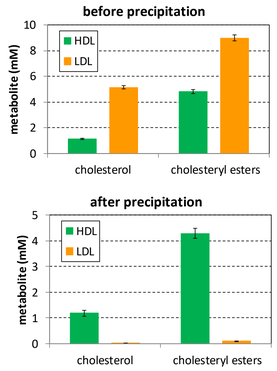



20,000 HepG2 cells per well were incubated overnight in the absence or presence of 0.3 mM BSA-bound linoleic and oleic acids to induce normal or steatoic conditions. Cells were washed with PBS and assayed with the triglyceride detection assay.

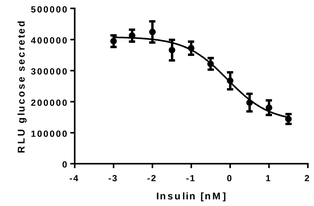



## 7. Cholesterol and Cholesteryl Esters in Lipoproteins

Human High Density Lipoprotein (HDL, 10 mg/ml) and Human Low Density Lipoprotein (LDL, 5 mg/ml) were purchased from Kalen Biomedical, LLC. Because of the high amounts of lipid and our assay's ~ 80 uM limit of linearity, samples were diluted 4000-fold prior to assay. Samples in the lower graph were pulled from the supernatant after treatment with an LDL Precipitation Buffer (Sigma).

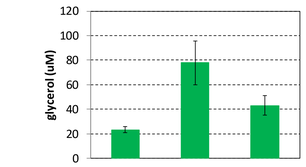



## 8. Insulin Action: Gluconeogenesis and Lipolysis




Microtissues (InSphero) formed from 2000 iCell® Hepatocytes 2.0 (CDI) were washed and incubated with 10 mM lactate, 2 uM forskolin, and a titration of insulin for 6 hours. An aliquot of media was then assayed with the glucose detection ssay.

3T3-L1 MBX adipocytes were washed and treated for 90 minutes with different combinations of isoproterenol (25 nM) and insulin (150 nM). An aliquot of media was then assayed with the glycerol detection assay.




| | | | |
|---|---|---|---|
| Isoproterenol | - | + | + |
| Insulin | - | - | + |

## 9. Conclusions

A variety of metabolites can be measured in a variety of sample types with these bioluminescent detections assays.

| Metabolites | Sample Types |
|---|---|
| **NAD(P)/NAD(P)H** | cancer cells |
| **glucose** | primary cells |
| **lactate** | stem cells |
| **glutamine** | immune cells |
| **glutamate** | 3D microtissues |
| **2DG6P** | bacteria |
| glycerol/triglyceride | yeast |
| cholesterol/cholesteryl esters | |
| branched-chain amino acids | |



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