Bioluminescent Assays for Investigating Insulin Action and Steatosis

Michael P. Valley, Donna Leippe, Natasha Karassina, James J. Cali, and Jolanta Vidugiriene

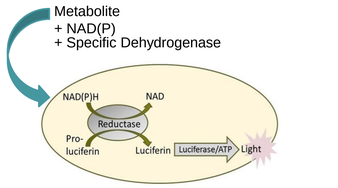
Promega Corporation, 2800 Woods Hollow Rd, Madison, WI 53711

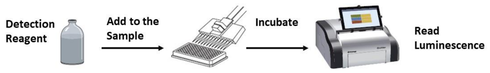
Abstract # 2786; Poster # P399

1. Bioluminescent Metabolite Detection Assays

We have developed a core bioluminescent technology that couples specific metabolite dehydrogenases to the production of NAD(P)H and the generation of light.

Metabolites
Lactate
Glucose
2-Deoxyglucose
Glycogen
Glutamine
Glutamate
BCAA
Glycerol
Triglycerides
Cholesterol
Cholesterol Esters

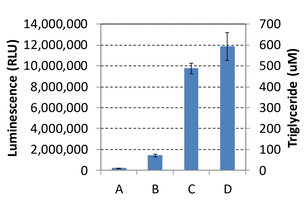




2. Triglyceride Accumulation During Adipocyte Differentiation

Oil Red O Staining

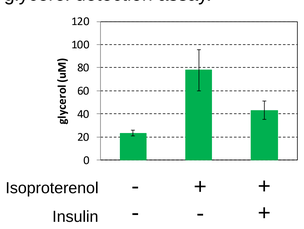
Triglyceride Detection Assay



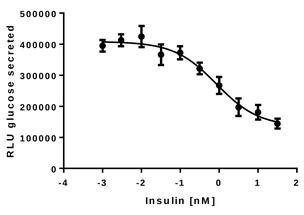
A – fibroblasts (3T3L1-MBX)
B – differentiation stage 1
C – differentiation stage 2
D – mature adipocytes

3. Inhibition of Lipolysis and Gluconeogenesis by Insulin

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.

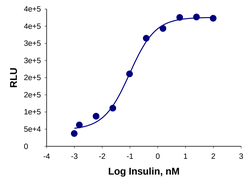


Microtissues (InSphero) formed from 2000 iCell® Hepatocytes 2.0 (CDI) were washed and incubated with 10 mM lactate, 2 uM forskolin, and a titration of insulin for 6 hours. An aliquot of media was then assayed with the glucose detection assay.

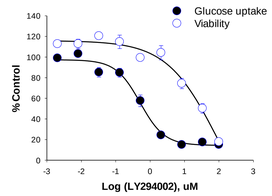


4. Stimulation of Glucose Uptake in Adipocytes by Insulin

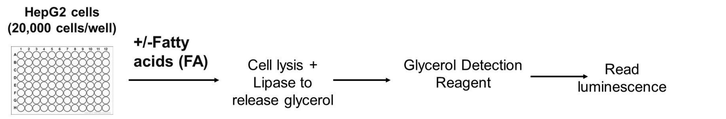
Insulin stimulates translocation of GLUT4 in 3T3-L1 MBX adipocytes. This results in a 10-fold increase in glucose uptake as measured with our bioluminescent assay with an EC_{50} of 0.1 nM.



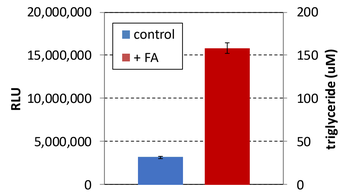
Insulin stimulated glucose uptake could be inhibited by the PI3K inhibitor LY294002. Cells were treated with inhibitor for 30 min and then 100 nM insulin for 1 hr. Viability and glucose uptake were then measured, yielding IC_{50}'s of 88 µM and 0.5 µM, respectively.



5. HepG2 as a model for NAFLD

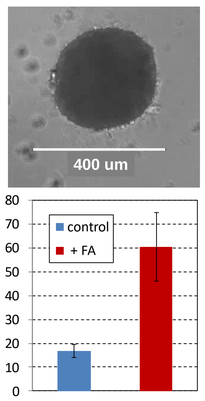


20,000 HepG2 cells per well were incubated overnight in the absence or presence of 0.3 mM BSA-bound linoleic and oleic acids to induce normal or steatoic conditions. Cells were washed with PBS and assayed with the triglyceride detection assay.



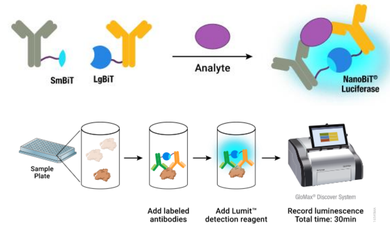
6. Hepatospheroids as a model for NAFLD

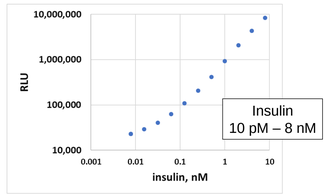
1,500 3D-qualified human hepatocytes were seeded in round bottom 96-well ultra low attachment plates in 200 µl of plating media and allowed to form spheroids over 5 days. The spheroids were fed with maintenance media (with a reduced amount of insulin, 10 nM) for 2 weeks in the absence or presence of 0.3 mM BSA-bound linoleic and oleic acids to induce normal or steatoic conditions. Feeding was performed every 3-4 days by removing 100 µl media, washing with 100 µl PBS, and adding 100 µl of 2x maintenance media. Just prior to assay, the spheroids were washed 4x with 100 ul PBS to minimize extracellular glycerol.

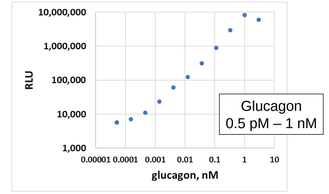


7. Lumit™ Immunoassays for Insulin and Glucagon Detection

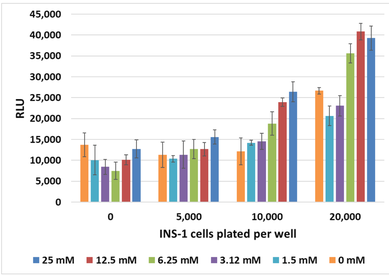
Lumit™ Immunoassays are based on NanoLuc® Binary Technology (NanoBiT®). In Lumit™ Immunoassays, antibodies are chemically labeled with the small and large subunits of NanoLuc® Luciferase, known as SmBiT and LgBiT, respectively. In the presence of an analyte, the two antibodies come into close proximity, allowing SmBiT and LgBiT to form an active enzyme and generate a bright luminescence signal.







8. Glucose-Stimulated Insulin Secretion (GSIS) in INS-1 cells



INS-1 rat insulinoma cells were plated at the indicated densities in 100 µl in the wells of a 96-well plate. After incubating for 3 days at 37°C, the cells were incubated in 100 µl GSIS Buffer containing glucose at the indicated concentration. Each glucose concentration was added to duplicate wells. The GSIS sample was collected after 1 hr at 37° and 10 µl was assayed in duplicate in 384-well plates.

9. Conclusions

Bioluminescent Metabolite Detection Assays
- Can measure cellular responses to insulin such as decreases in lipid catabolism, increases in glucose uptake, and decreases in glucose production
- Compatible with various sample types such as media, cell lysates, and 3D microtissues

Bioluminescent Lumit™ Immunoassays
- Can measure insulin and glucagon with picomolar sensitivity and large linear range
- Can measure secreted insulin in functional GSIS tests

Corresponding author: mike.valley@promega.com