A high throughput, mix and read solution for the study of metabolic diseases

HTRF detection of GLP-1

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Venenum Biodesign

Discovery is our Mission

LLC Founded May 11, 2009

- Full operations commenced in Dec 2010
- Funded through founder / CEO's diagnostics business

31 employees (biology & chemistry)

Established with the mission of improving patients' lives through the discovery of therapeutic compounds at our novel biology platforms

Our goals are to:

- Advance our discoveries to products through partnership
- Develop collaborations around our core capabilities

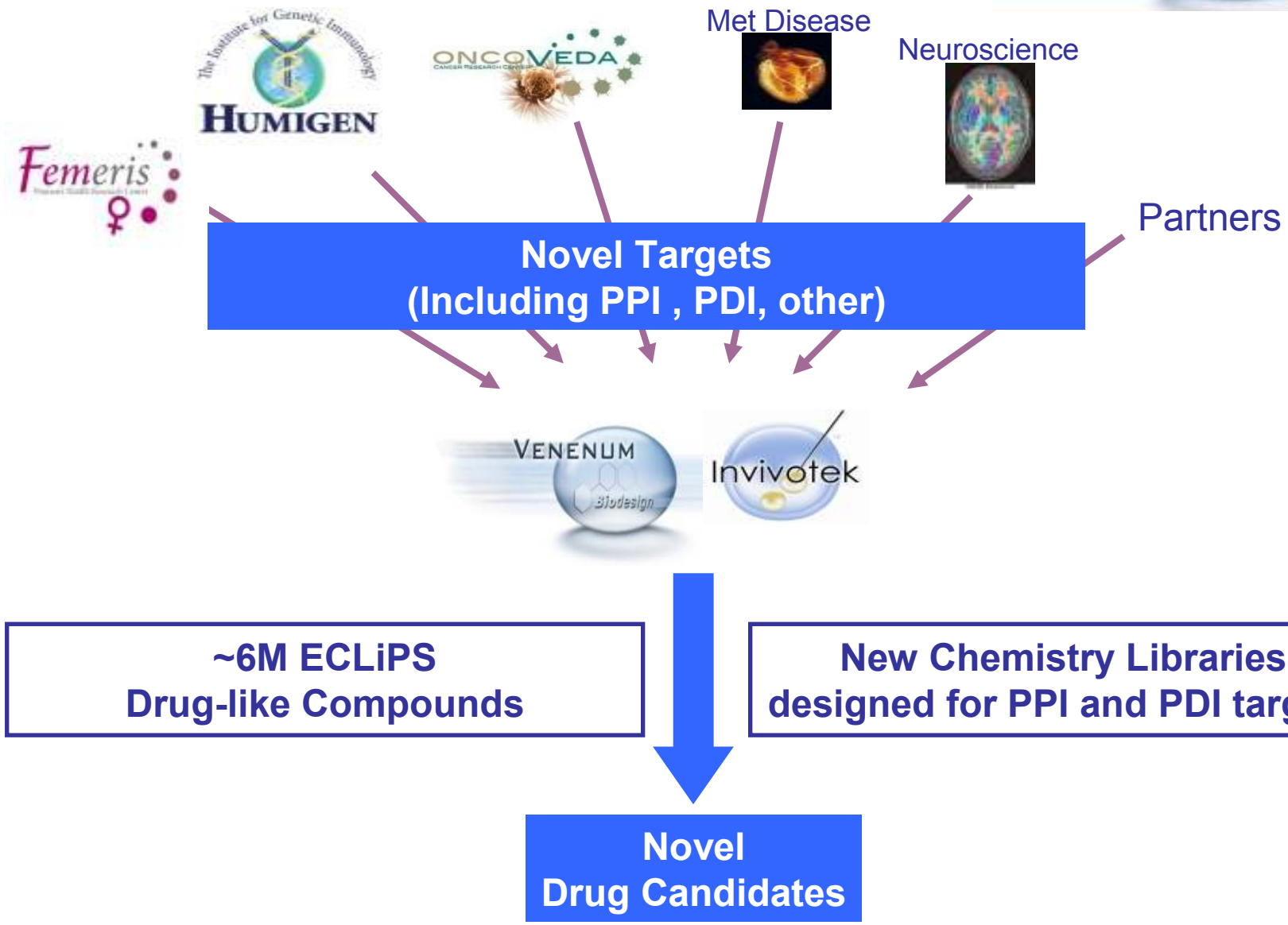
GENESIS Biotechnology Group

- Drug Discovery
- Genetic Immunology Research Center
- Medical Diagnostic Laboratories
  - Infectious Diseases and Women's Health Research Center
  - Cancer Research Center
- Plastic Manufacturing
- Wholesale Research Equipment and Labware
- Vivarium

UMDNJ SCHOOL OF OSTEOPATHIC MEDICINE
University of Medicine & Dentistry of New Jersey

- *Affiliation with University of Medicine & Dentistry of New Jersey*

Mining Novel Biology with New Chemistry



TGR5 Overview

Human TGR5 ≡ **T**akeda **G**-protein coupled **R**eceptor **5**

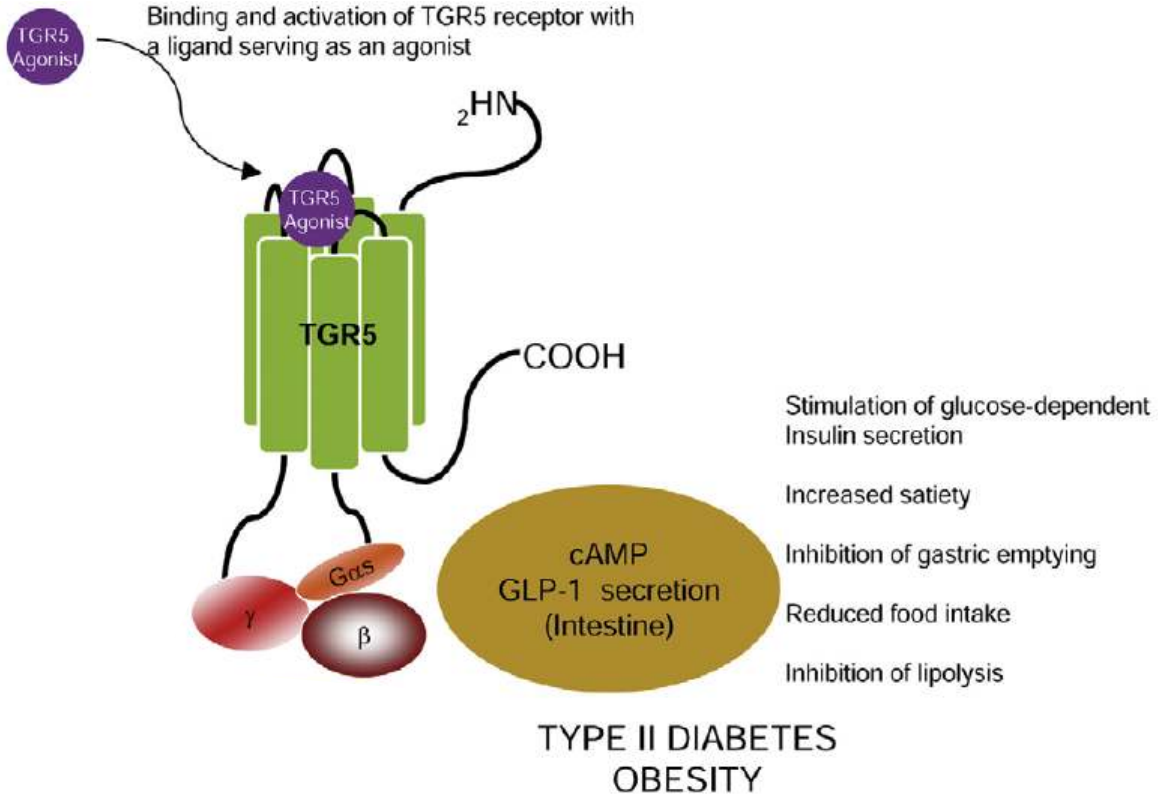
- GPBAR1 ≡ G-protein coupled Bile Acid Receptor 1
- GPR131, BG37, hGPCR19, AXOR109, M-BAR, MGC 40597

TGR5

- De-orphaned as the Bile Acid Receptor in the 2002 timeframe
- Class A GPCR – G_s coupled
- 330 amino acids – encoded by a single exon

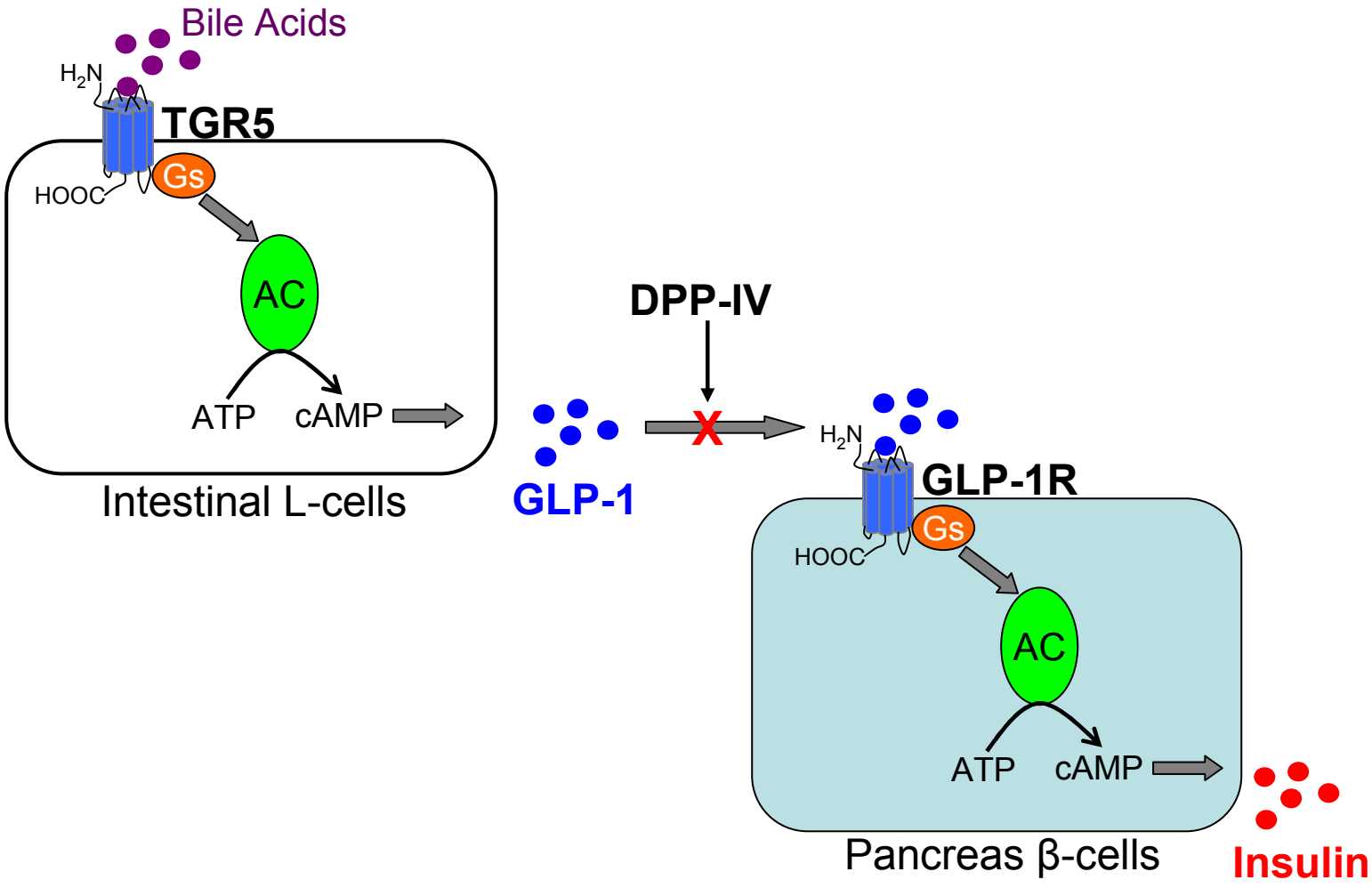
- Ubiquitously expressed with high expression in liver, intestine, brown adipose tissue and spleen

TGR5 Affects – Potential Therapeutic Areas



Drug Discovery Today

Effect of TGR5 Agonism



TGR5 Program Status

uHTS Survey of Venenum's ECLiPS Collection

- Used CHO Cell Line to detect cAMP increase
- Found Multiple Series to start Hit-to-Lead Program

Hit-to-Lead Progress

- One series in particular has made significant progress
- Very Potent full agonists in cAMP assays
- Has both human and mouse activity (vs. cAMP)

GLP-1 Detection is now crucial to the progress of the program

- While cAMP is nice and easy, GLP-1 is the final endpoint
- The ability of a compound to produce GLP-1 in an *in vitro* assay is the final filter before testing in an *in vivo* system.

GLP-1 Detection

Current Gold Standard in Industry is the ELISA

- VERY labor intensive – requires washing
- Requires Planning and Time
- Locked into 96-well format – including their plate
- Expensive per well ($5/well)

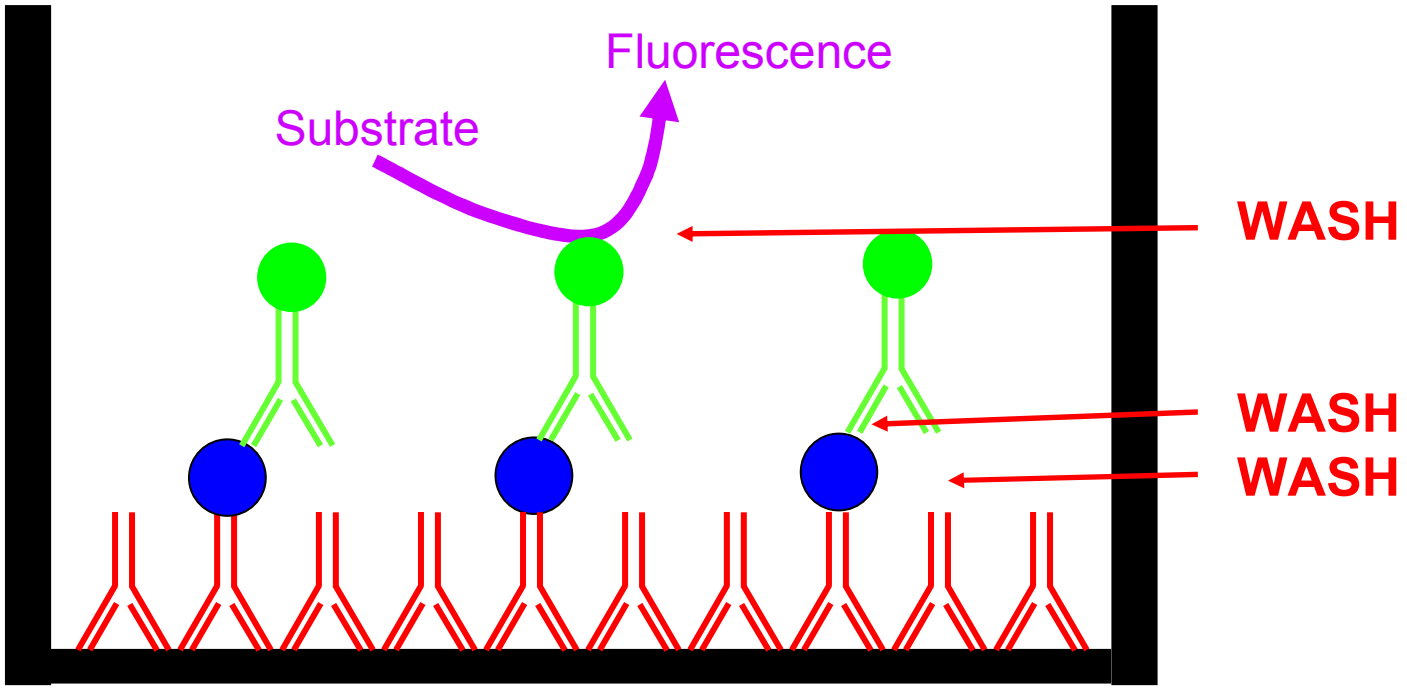
Goals for a GLP-1 Detection Kit

- Concentration-dependent curves, 4 or 5 concentrations – not single point
- **LOTS** of compounds
- Multiple *in vitro* cell lines (human, mouse, etc…)
- *In vivo* studies – directly from mouse blood

- Prefer a 384-well or low-volume 384-well format

New HTRF Kit from CisBio could achieve my goals

ELISA GLP-1 Schematic

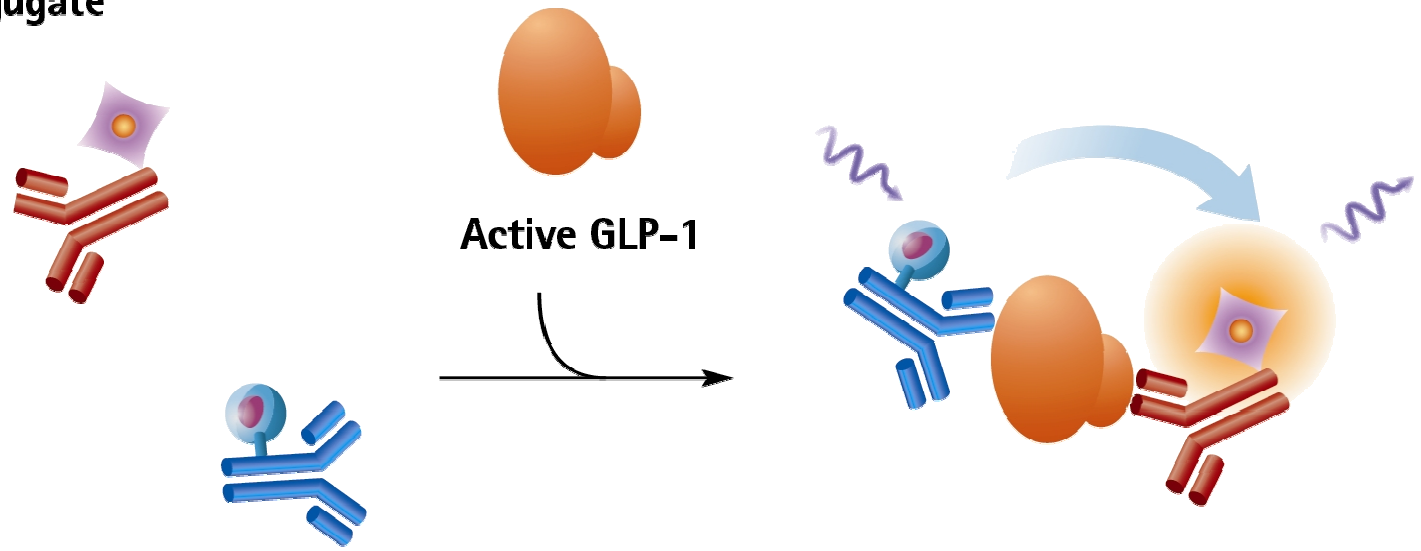


Sandwich ELISA – 2 antibody System

96-well Plate with antibody pre-coated onto surface

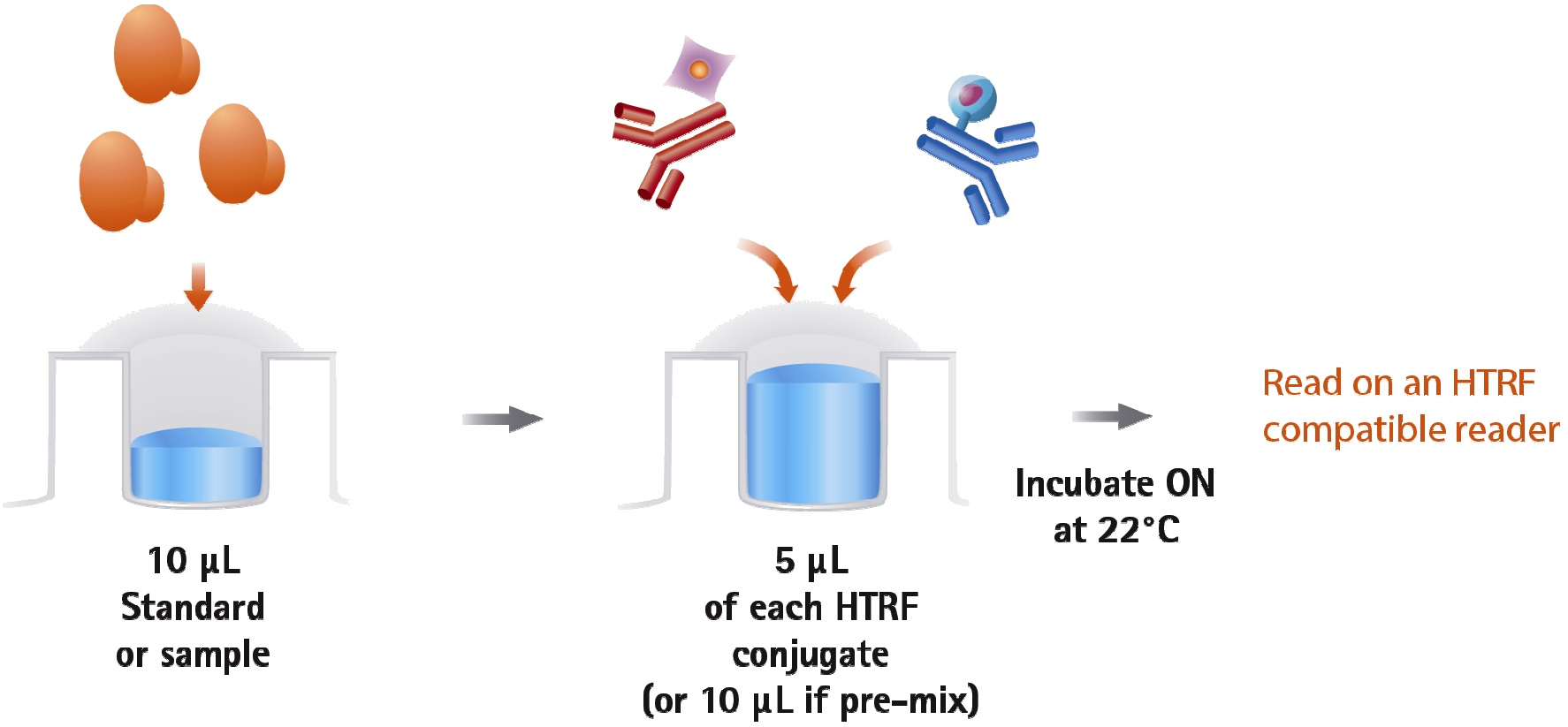
CisBio GLP-1 Kit with HTRF Detection

Pab Anti-GLP-1 C-terminal
d2 conjugate



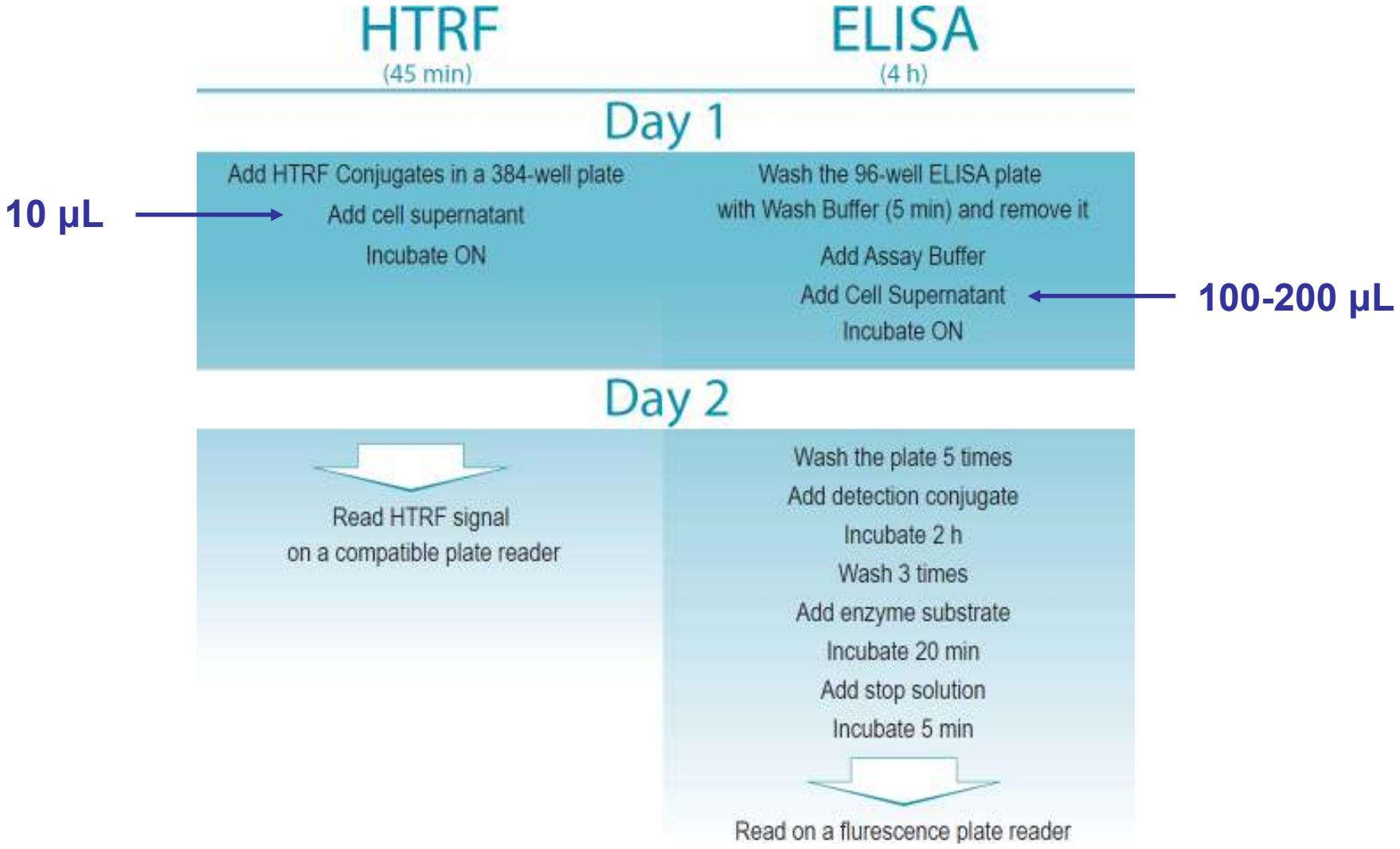
Mab Anti-GLP-1(7-12) fragment
Terbium Cryptate conjugate

The HTRF Method

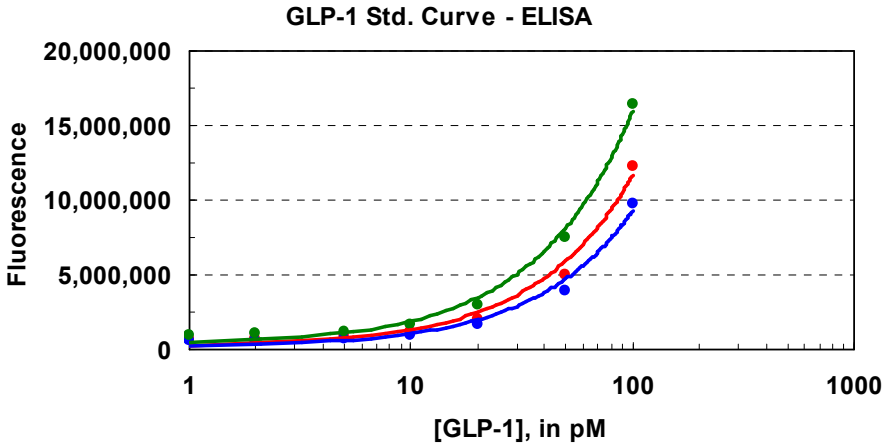


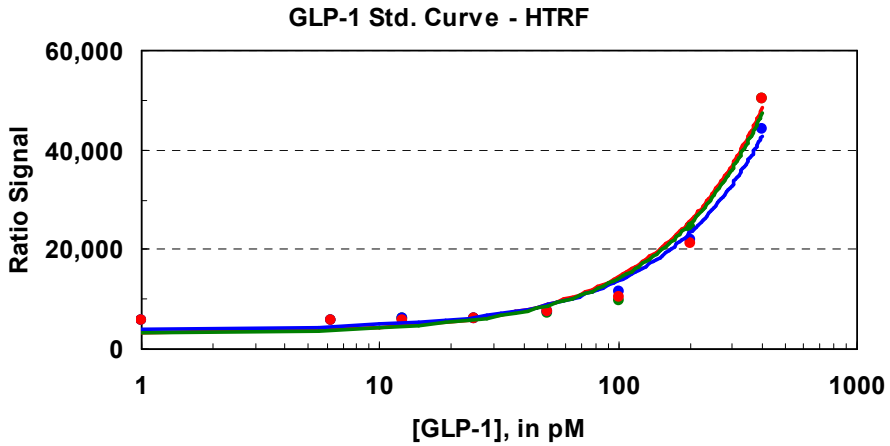
Method Comparison

Both Techniques require stimulating cells with compound beforehand



Standard Curve Comparison





PerkinElmer Envision is the instrument of choice for detection for both methods.

- ELISA Exc @ 355 nm
  Em @ 460 nm
- HTRF Exc @ 337 nm (laser)
  Em @ 665 & 615 nm

Standard Curve Points

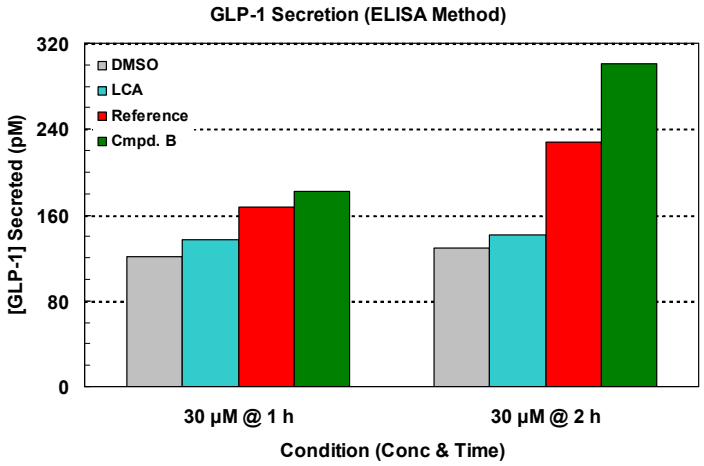
- ELISA is 2 to 100 pM
- HTRF 6.25 to 400 pM

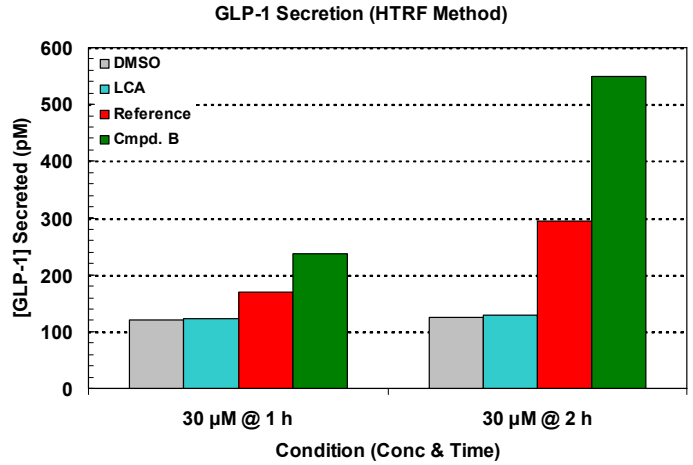
Limit of Detection (non-statistical)

- ELISA is ~2-3 pM GLP-1
- HTRF is ~10-15 pM GLP-1

Detection from NCI-H716 Cells

NCI-H716 is a human intestinal cell line with endogenous levels of TGR5 expressed and secretes GLP-1 when stimulated,





ELISA and HTRF methods have always yielded the same answer as to which compounds are active, but the exact amount of GLP-1 has slightly differed.

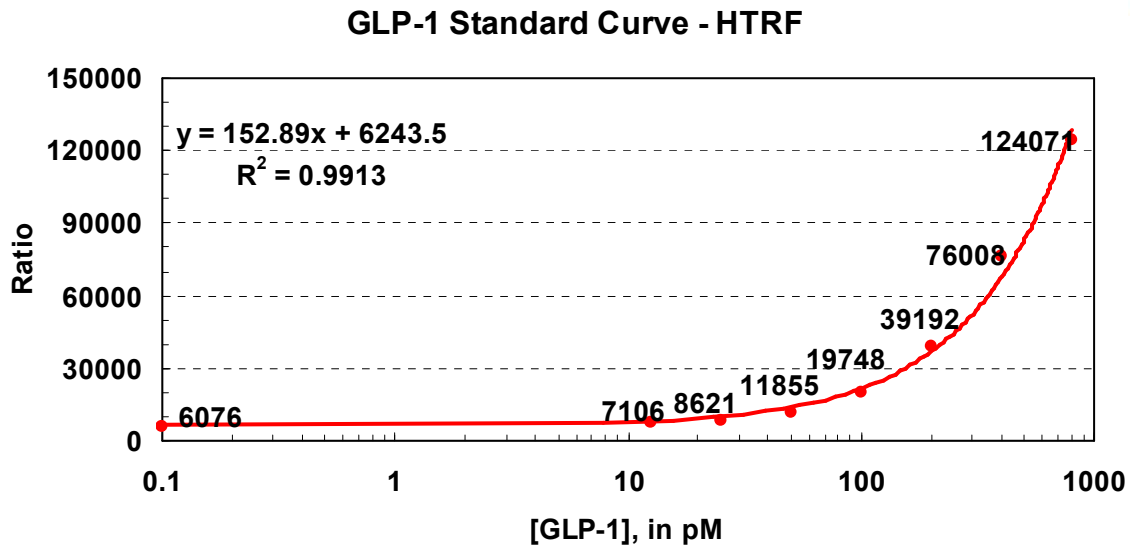
S:B for HTRF was better than ELISA

Basal Level in NCI-H716 Cells

- High - 10s to 100s pM
- Looking to reduce basal level

Currently, levels of GLP-1 in the cell based assay is more in line with linear range of the CisBio HTRF Kit.

Longer HTRF Standard Curve



Standards more in line with cell results

- Useful though up to 20-fold window

Ratio numbers are almost identical to standard run month previous.

Conc.	11/21/2012	12/20/2012	1/11/2013
0	5,635	6,076	5,837
12.5	6,806	7,106	7,026
25	8,503	8,621	8,752
50	11,798	11,855	12,846
100	19,089	19,748	22,661
200	35,942	39,192	42,612
400	73,368	76,008	81,293
800	120,824	124,071	121,242

Order of Addition

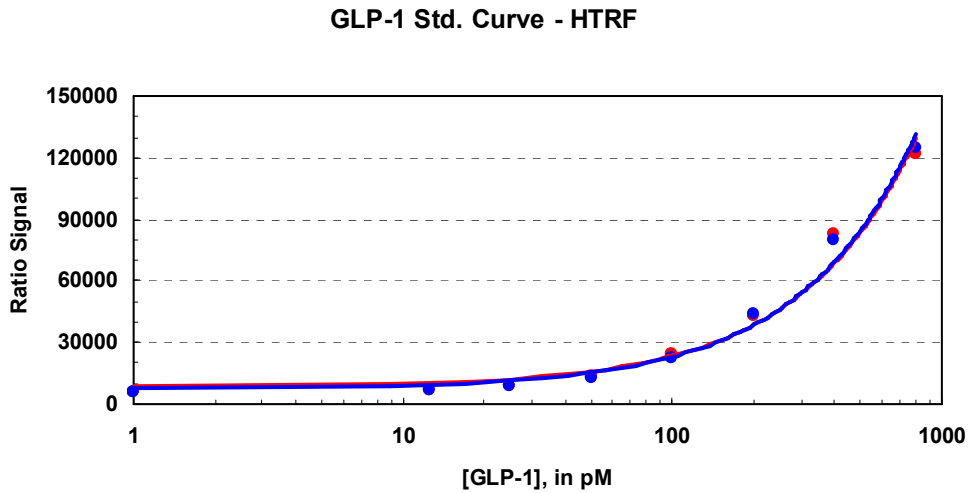
In HTRF Kit, you have a choice

- Add in your cell supernatent first
- Add in your detection reagents first - recommended to us.

Could be a key piece of advice – especially at the lower GLP-1 concentrations.

Tested this:

- We have not seen a difference in signal or sensitivity to date



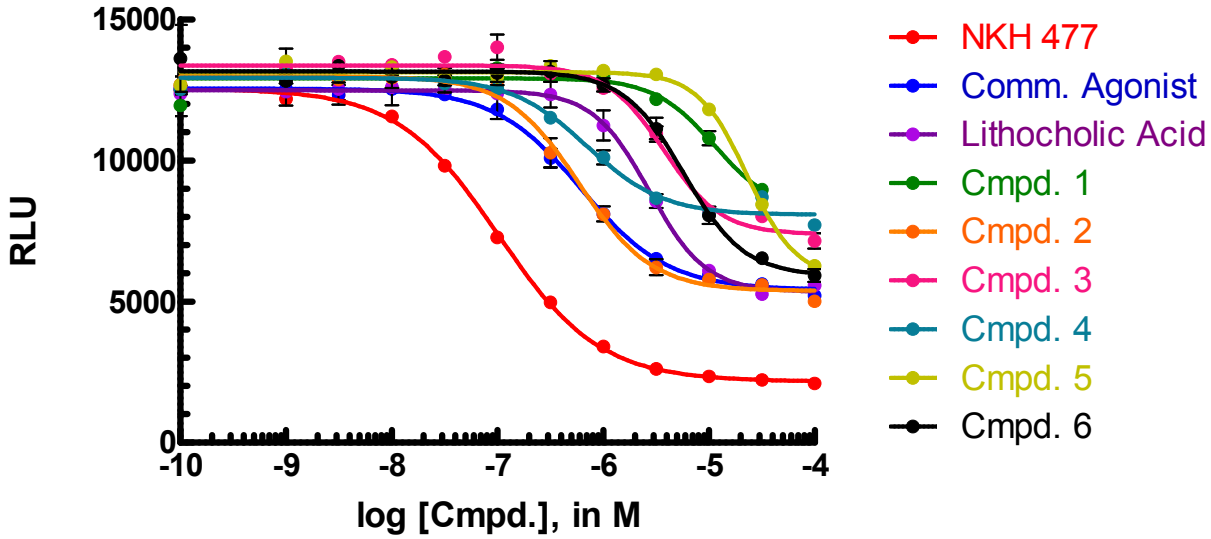
NCI-H716 Cells

NCI-H716 Cells are the most problematic part of assay

- GLP-1 Secretion is inconsistent experiment to experiment
- Suspension Cell Line, but GLP-1 assay prefers the cells to be settled

The cAMP Detection is very steady assay

- Uses the CisBio Femto cAMP Kit
  - Low S:B (~2), but it works
  - Suspension Set-Up – cells harvested directly from flask



NCI-H716 Cells Optimization

Using HTRF Kit to Optimize the Cell Handling

Lots of Parameters to examine

- Cell Number (5K to 100K per well)
- Suspension or Settled Cells
- Settling Time (1 day or 2 day)
- Media & Serum (which one, age, serum-starve, etc…)
- Incubation Time with the compounds
- Glucose Concentration (when do you add it?)
- Presence of IBMX and DPPIV Inhibitors Required?

Second Cell Line is now available for testing – STC-1

- Intestinal Cell Line from Mouse
- Endogenous TGR5 levels
- Adherent !!

In vivo Detection

Mouse Models Used

- Brand new facility at GBG – Invivotek
- Doing our initial experiments.

What are the basal levels of GLP-1 in blood?

Both the ELISA and HTRF yielded similar results

- Near the lower limit of detection
  - Calculation formula isn't really useful to calculate pM
  - Signal in most of the samples are slightly above "0" GLP-1 standard
    - Is it really GLP-1 or is the difference with sample?

Conclusions

Kit for HTRF GLP-1 works well

- Worked 1st time in lab
- GLP-1 Standard Curve is consistent experiment to experiment

More time spent on optimization of the cell line

- Using the HTRF Kit
- Cell line yielded inconsistent results
- Also looking to lower basal level of GLP-1 towards limit of detection
- Still Working On It !

Not sure if sensitive enough for *in vivo* mouse work

- Basal level of GLP-1 in mice is near or at limit of detection for kit
- New experiments are planned in Q1 where we are going to look at GLP-1 levels in stimulated vs. unstimulated mice.