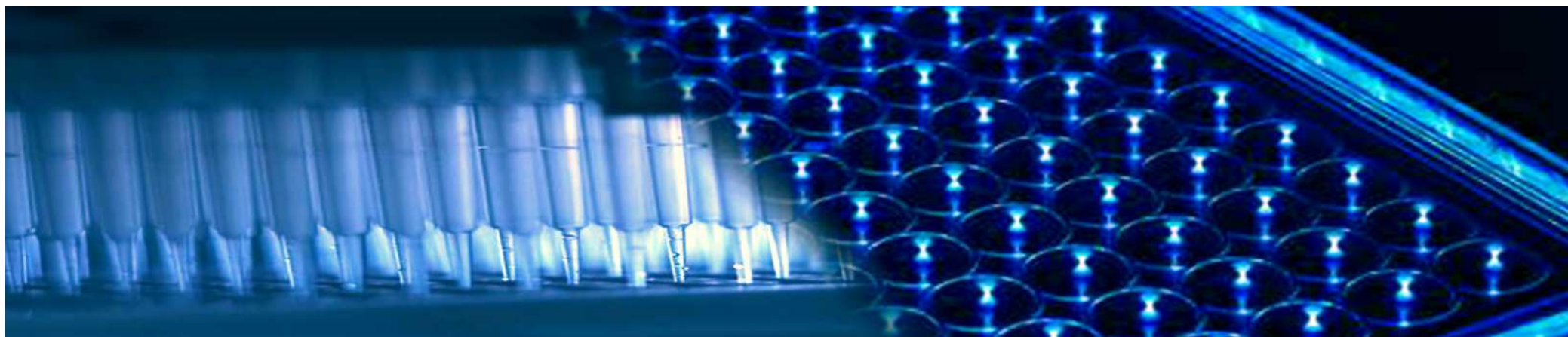


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# **Two GPCR Case Studies with the IP-One Eu and Tb Assay Kits**

*A Positive Modulator and an Inverse Agonist Project*

*Martin Graf, F. Hoffmann-La Roche*



# The Pharma Research Sites of Roche



Basel



Palo Alto



Penzberg



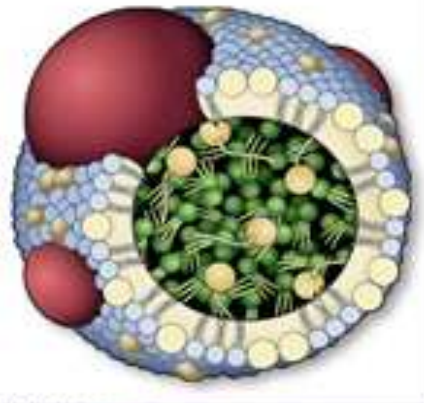
Nutley



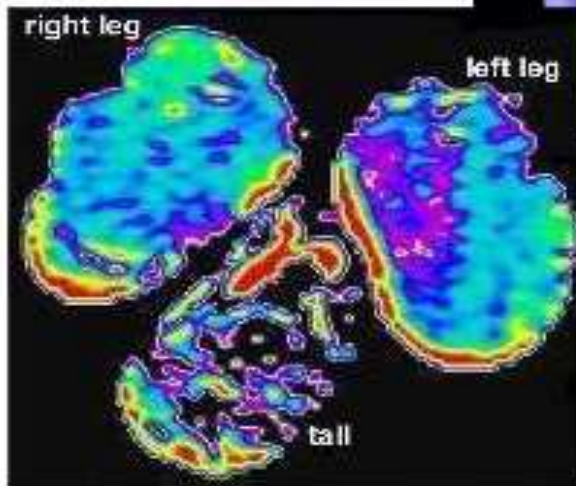
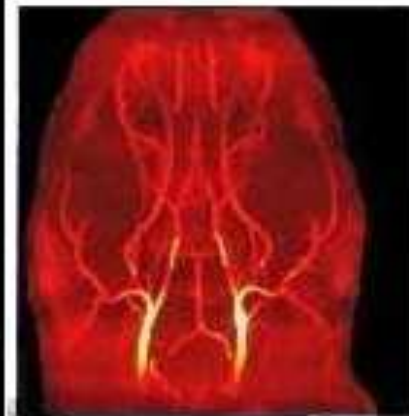
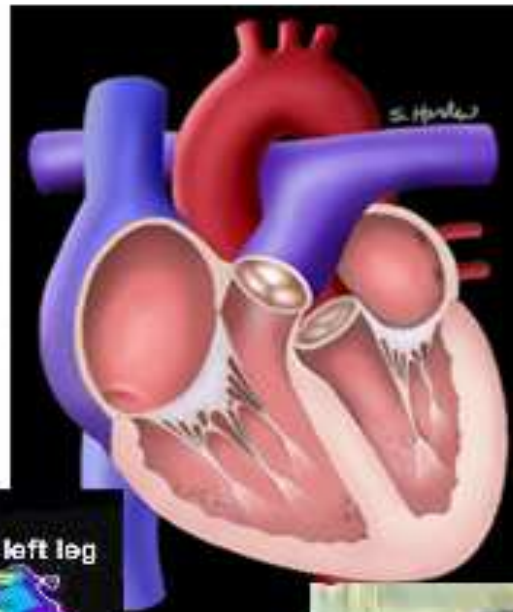
# Discovery Research at Roche Basel

## *Vascular & Metabolic Diseases*

***Dyslipidemia***



***Vascular Diseases***



***Diabetes***



***Obesity***

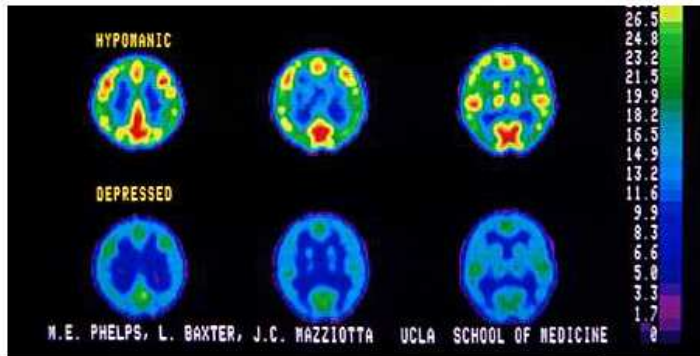
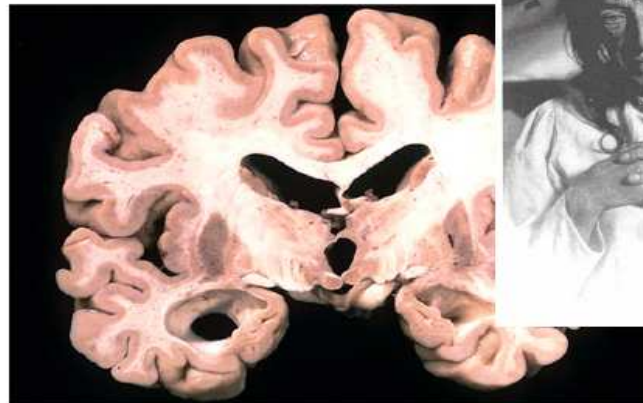
# Discovery Research at Roche Basel

## Neurosciences

### *Bipolar Disorder*

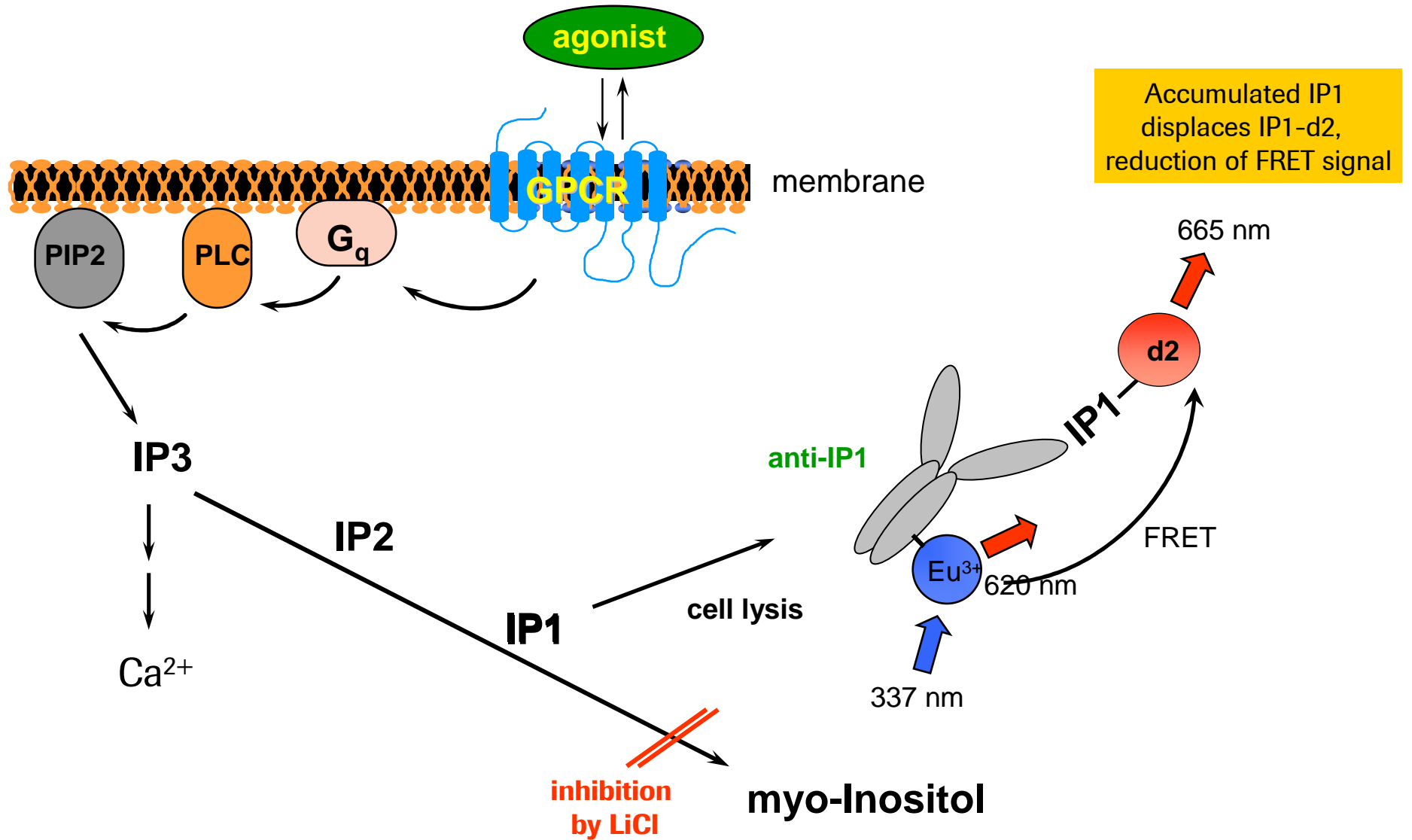


### *Alzheimer's Disease*

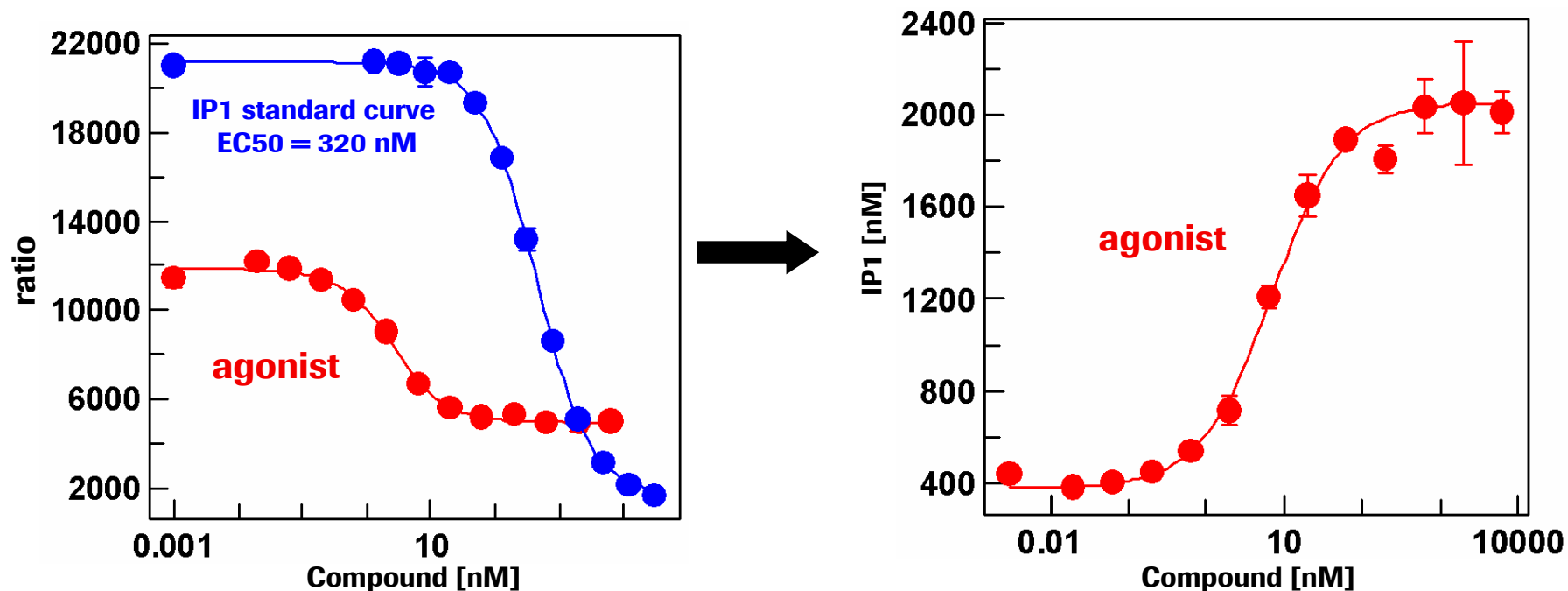


### *Depression*

# IP-One Assay Principle



# IP1 standard curve & agonist dose response



**Calculations:**  $Ratio = \frac{RFU_{665nm}}{RFU_{620nm}} * 10000$

$$DeltaF(\%) = \frac{Ratio_{sample} - Ratio_{neg}}{Ratio_{neg}} * 100$$

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## **GPCR1 - Positive Modulator Project**

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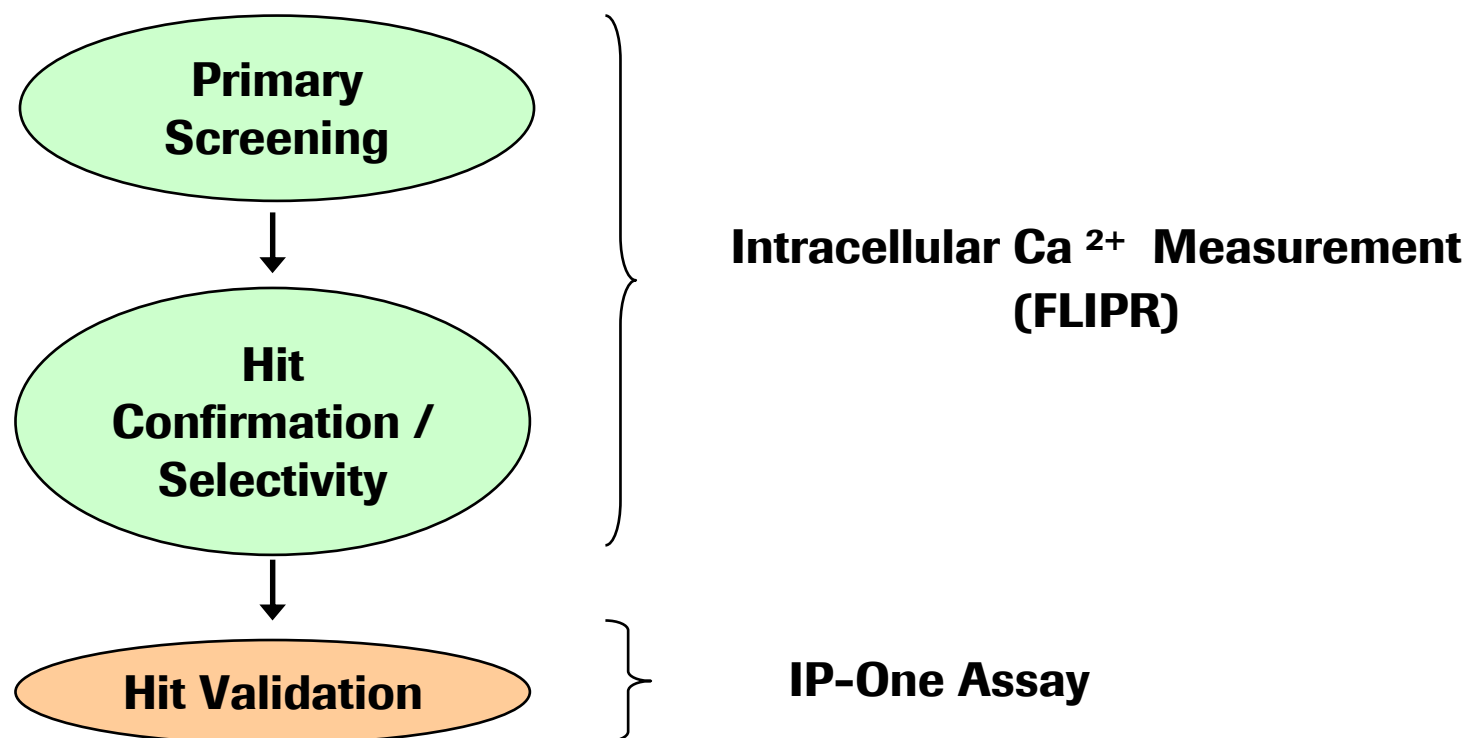
### **GPCR2 - Inverse Agonist Project**

### **First Results with the Terbium Kit**

### **Summary and Conclusion**

# Screening Cascade – GPCR1

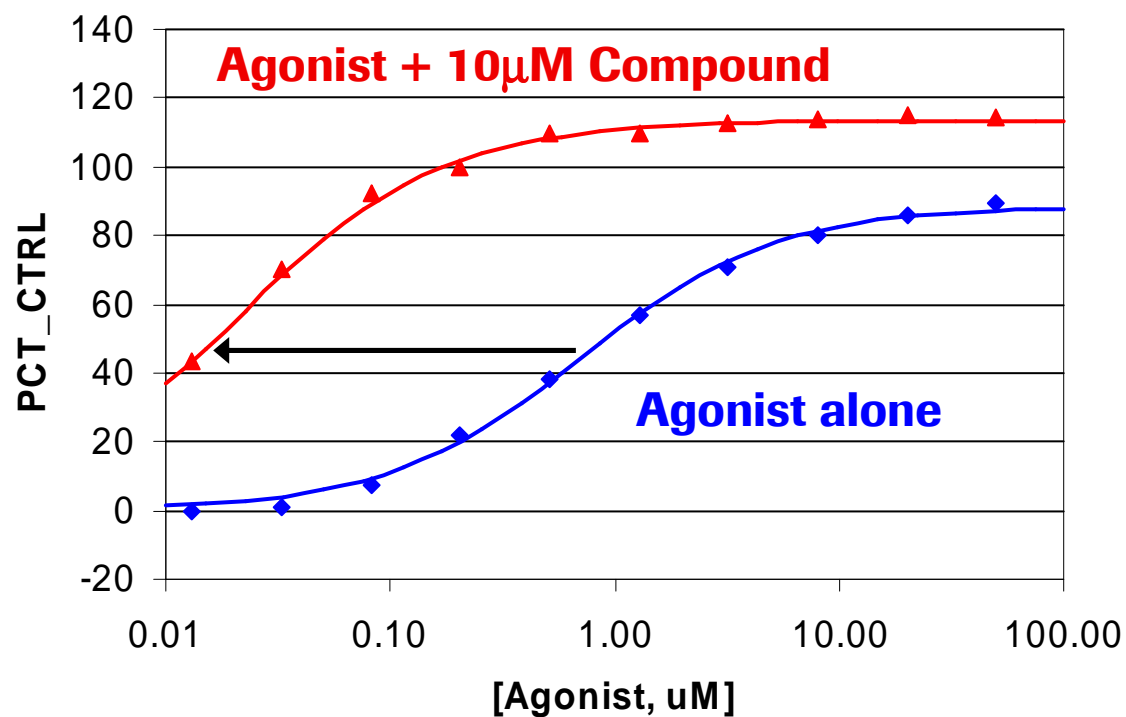
## *Positive Modulator Project*





# Agonist Shift Assay

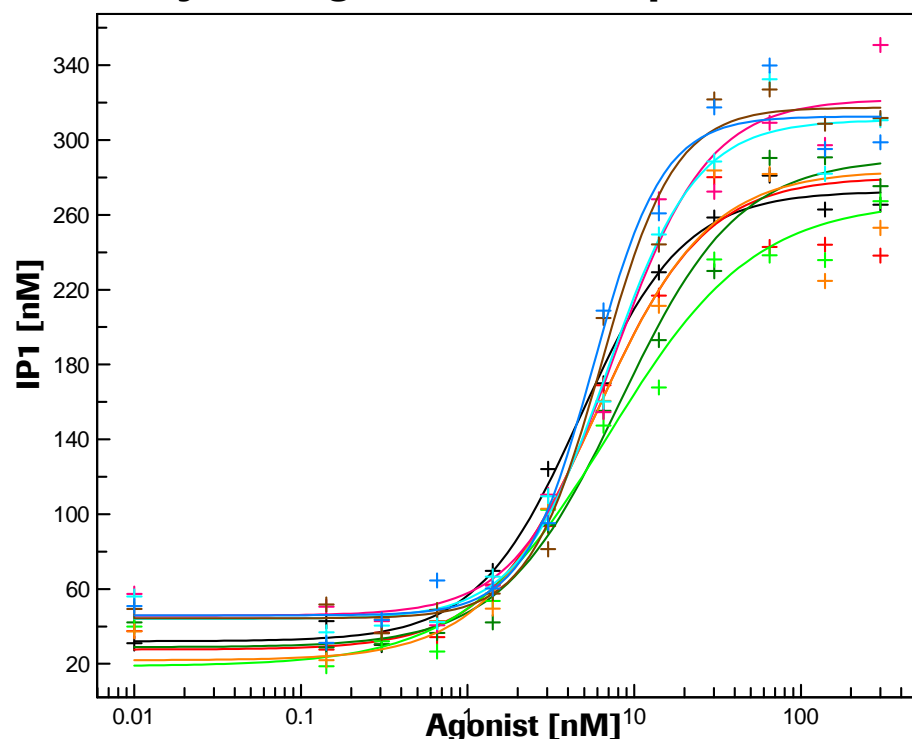
## *GPCR1 - Positive Modulator Project*



$$\text{Agonist Shift} = \text{pEC50}_{\text{Agonist}} - \text{pEC50}_{\text{Agonist+Compound}}$$

# Significance of the Agonist-Shift

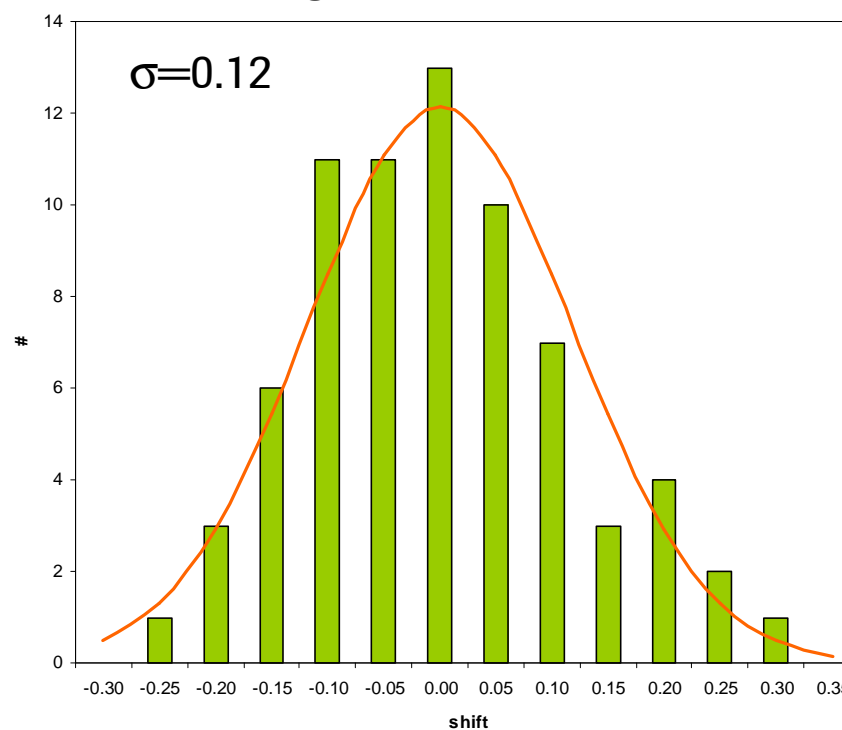
## Overlay of 9 Agonist Dose Response Curves



**IP-One:  $pEC_{50_{Ago}} = 8.22 \pm 0.09$  (n=9)**

**FLIPR:  $pEC_{50_{Ago}} = 8.66 \pm 0.13$  (n= 320)**

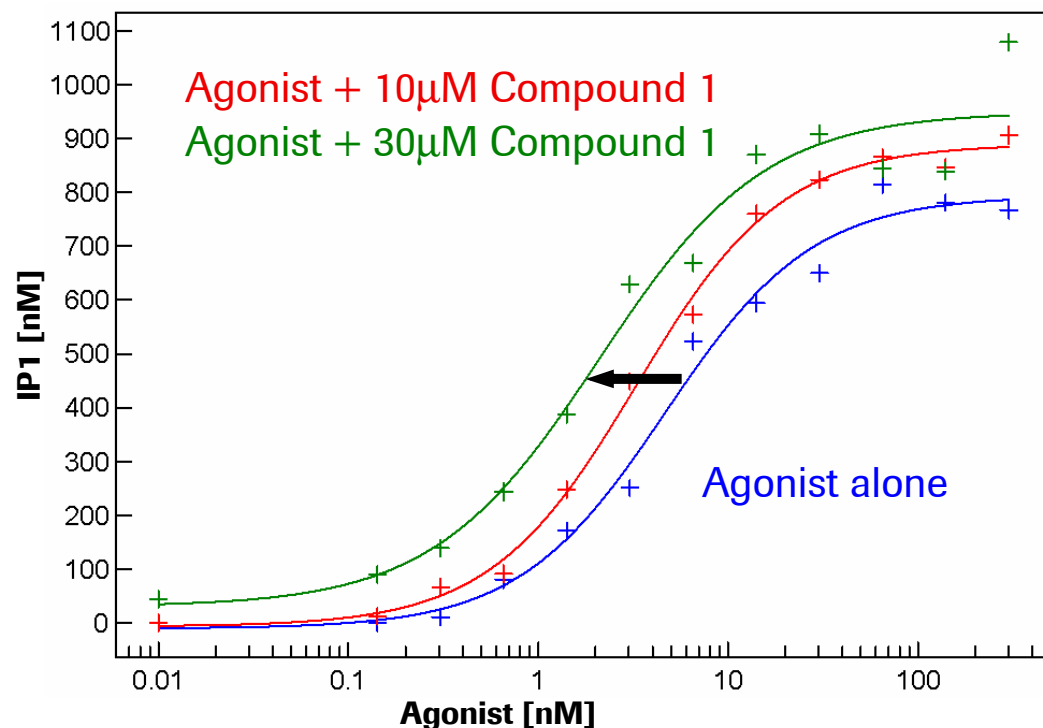
## Histogram of the shifts



shifts larger than  $2\sigma = 0.24$  are significant

(FLIPR  $2\sigma = 0.37$ )

# Agonist Shift Results of Some Hits



**$pEC_{50}^{\text{Agonist}} = 8.33$**

**Shift at 10µM = -0.15**

**Shift at 30µM = -0.35\*\***

## Comparison: Agonist Shifts FLIPR vs. IP-One

@ 10uM	Shift FLIPR	Shift IPone
Cmpd 1	-0.36	-0.15
Cmpd 2	-0.39	+0.34
Cmpd 3	-0.40	-0.32

**Validation in 2<sup>nd</sup> assay is important**



## **GPCR1 - Positive Modulator Project**

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## **GPCR2 - Inverse Agonist Project**

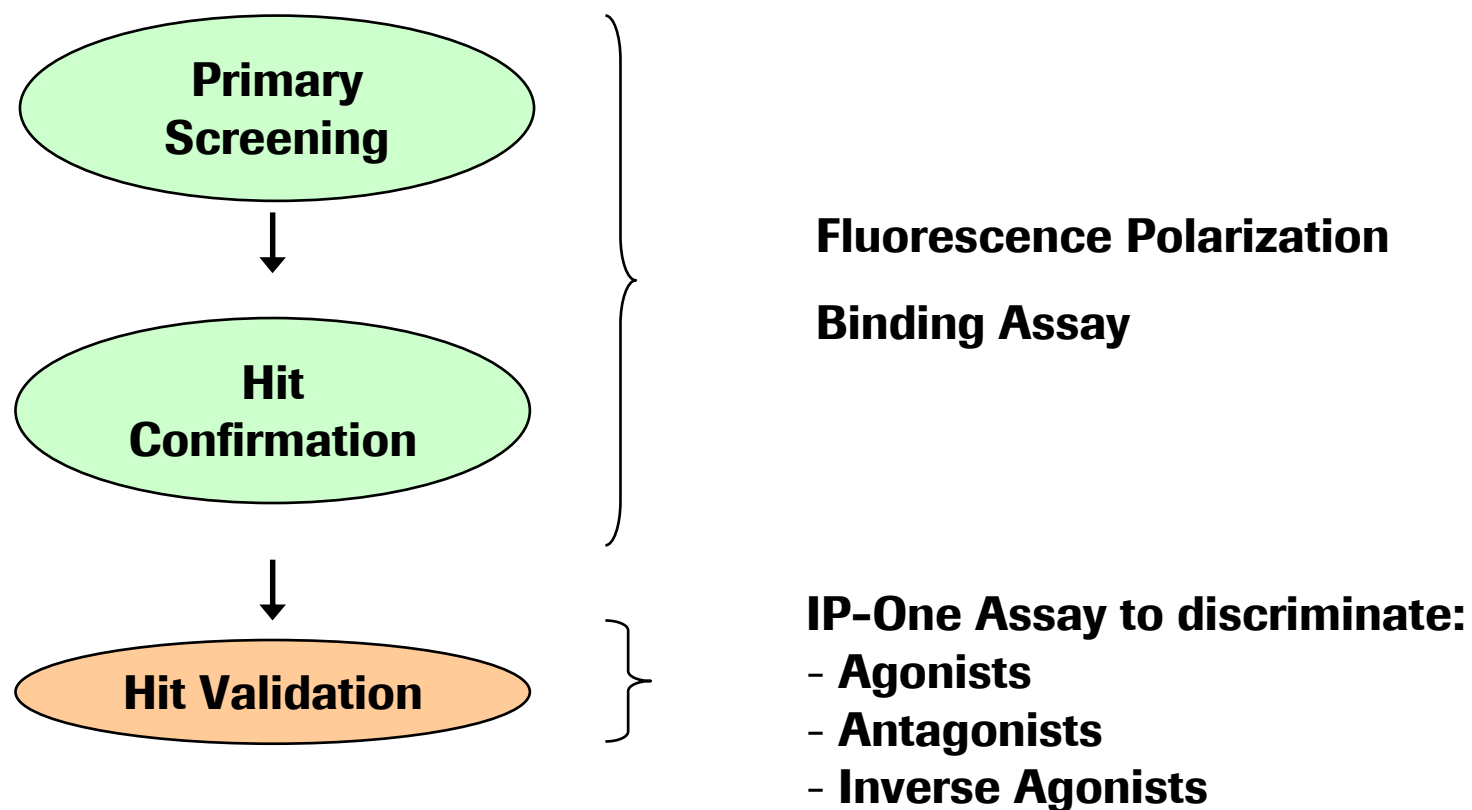
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## **First Results with the Terbium Kit**

## **Summary and Conclusion**

# Screening Cascade – GPCR2

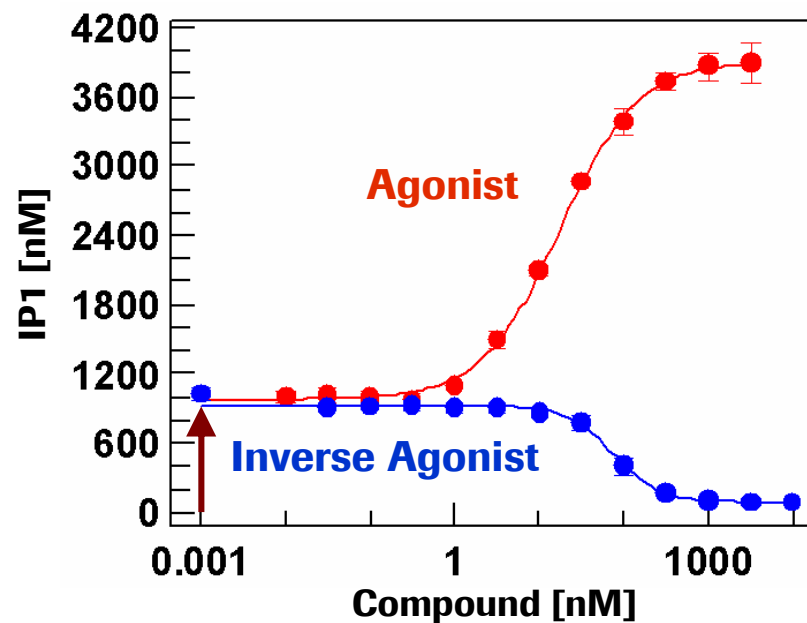
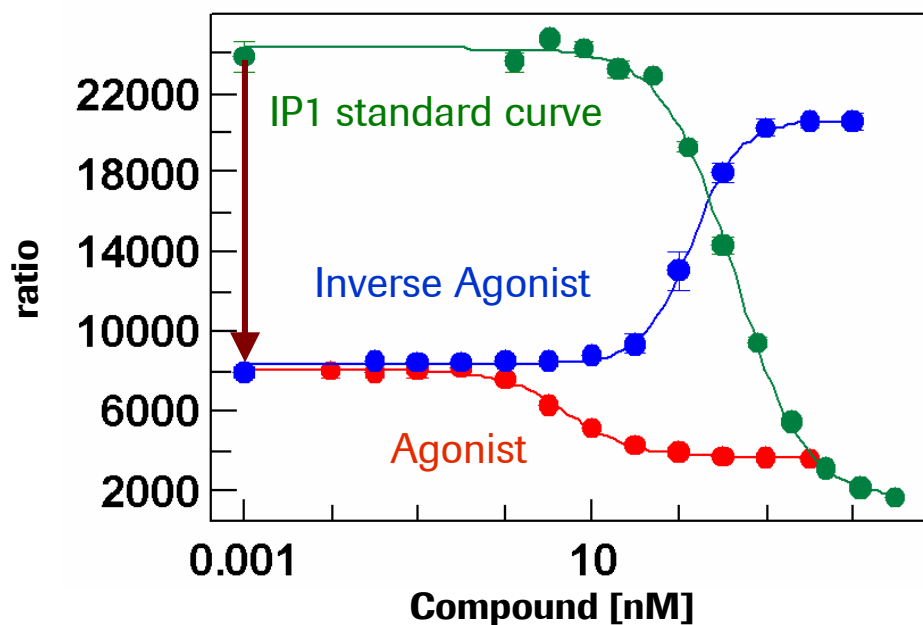
## *Inverse Agonist Project*





# Optimization of Agonist and inverse Agonist Mode (1)

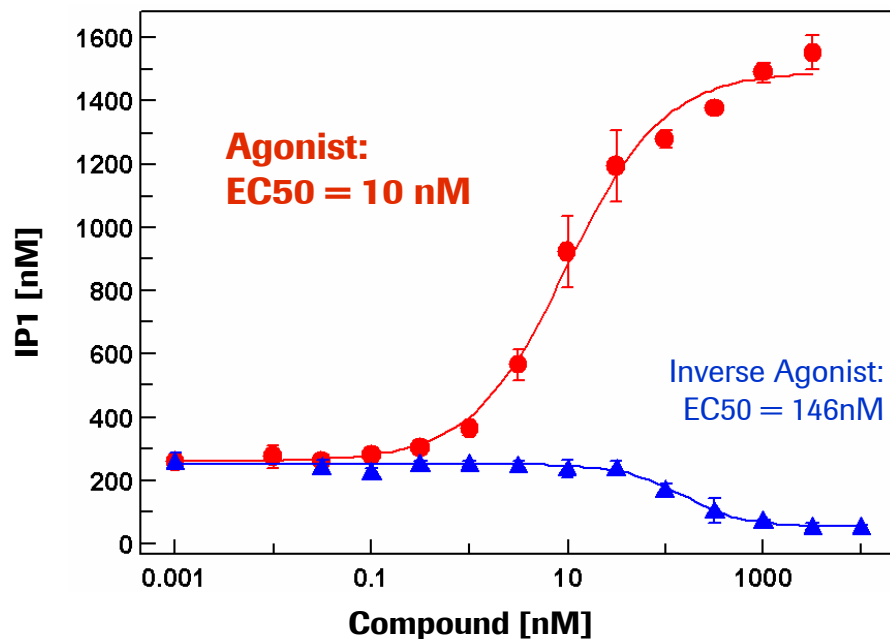
Conditions: 7'500 cells/well, 2h incubation



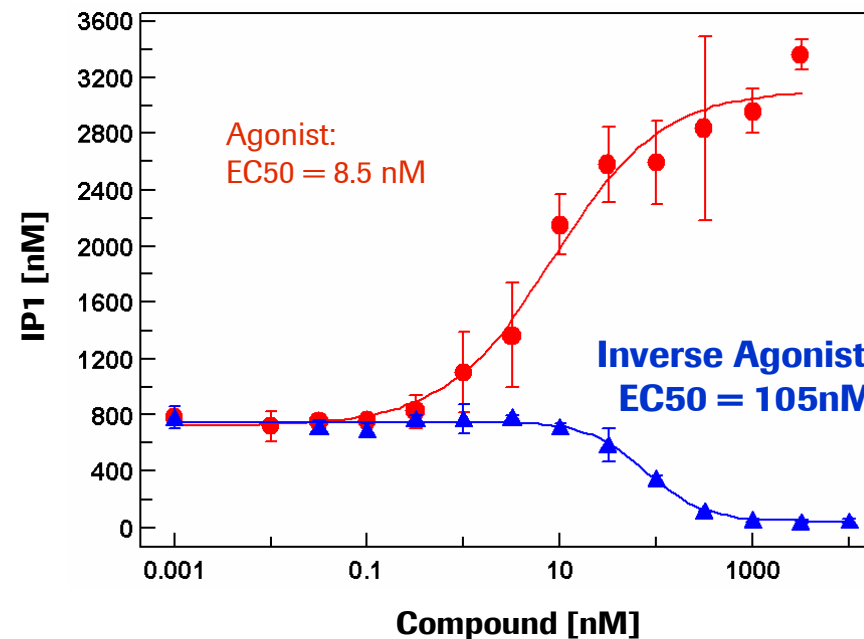
**constitutive activity**

# Optimization of Agonist and inverse Agonist Mode (2)

**Best Condition for Agonist Mode:  
7'500 cells/well, 1h incubation**

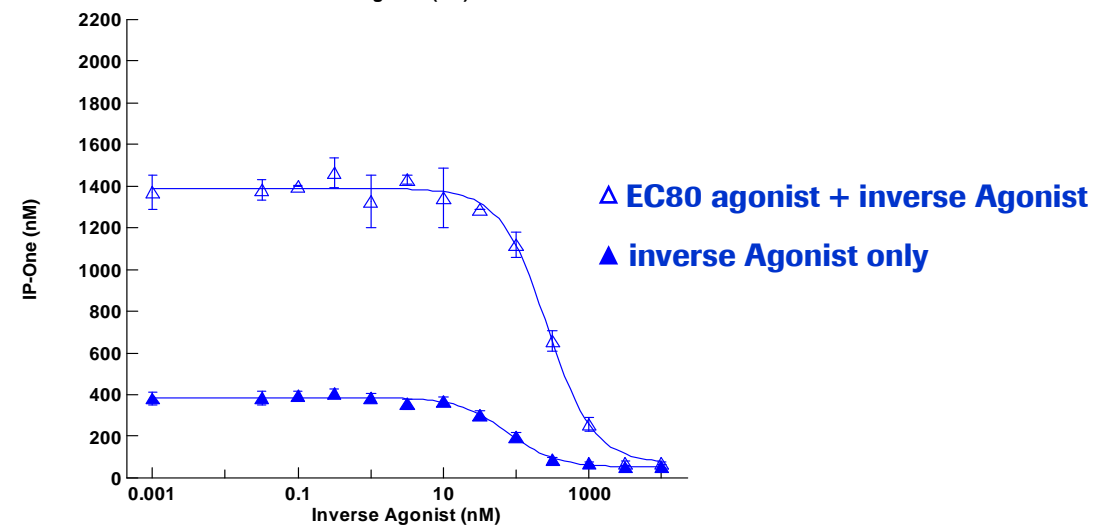
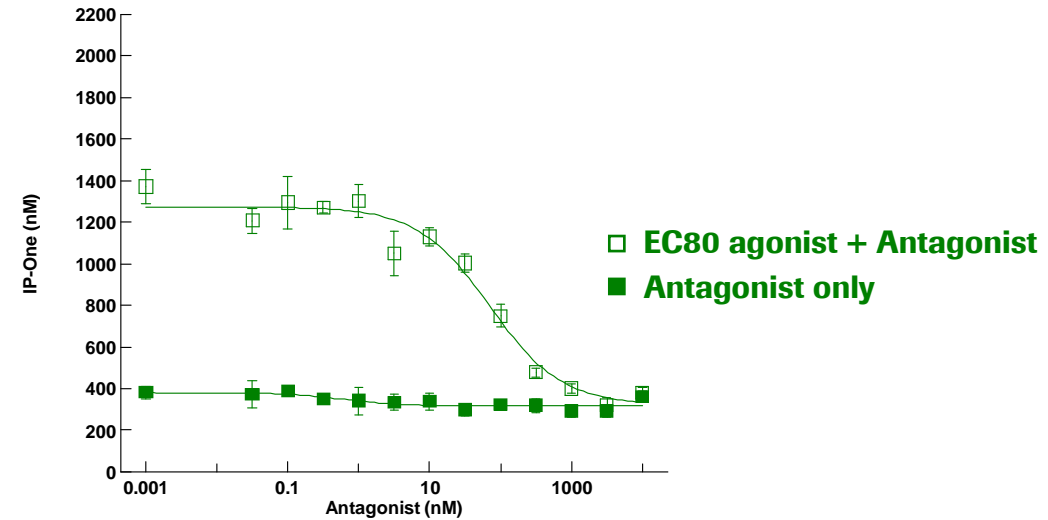
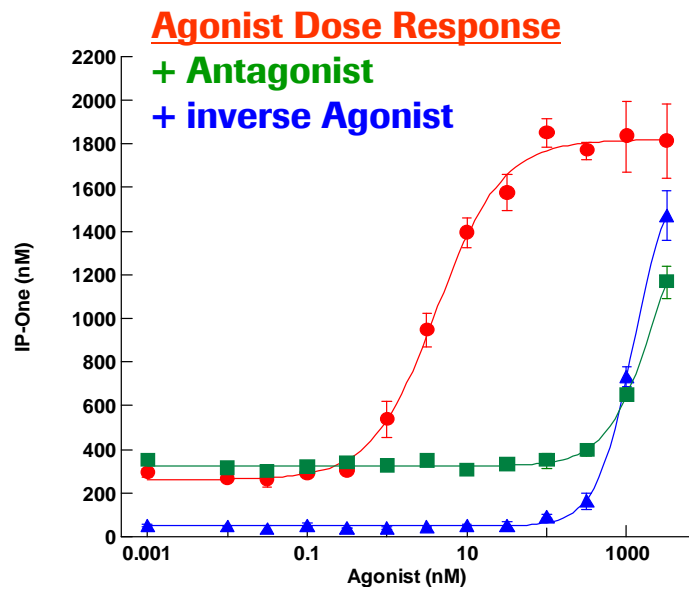


**Best Condition for inverse Agonist Mode:  
7'500 cells/well, 4h incubation**



Cell Number and Incubation time Optimization  
in order to cover dynamic range of the standard curve

# Comparison of an Antagonist and an Inverse Agonist





**GPCR1 - Positive Modulator Project**

**GPCR2 - Inverse Agonist Project**

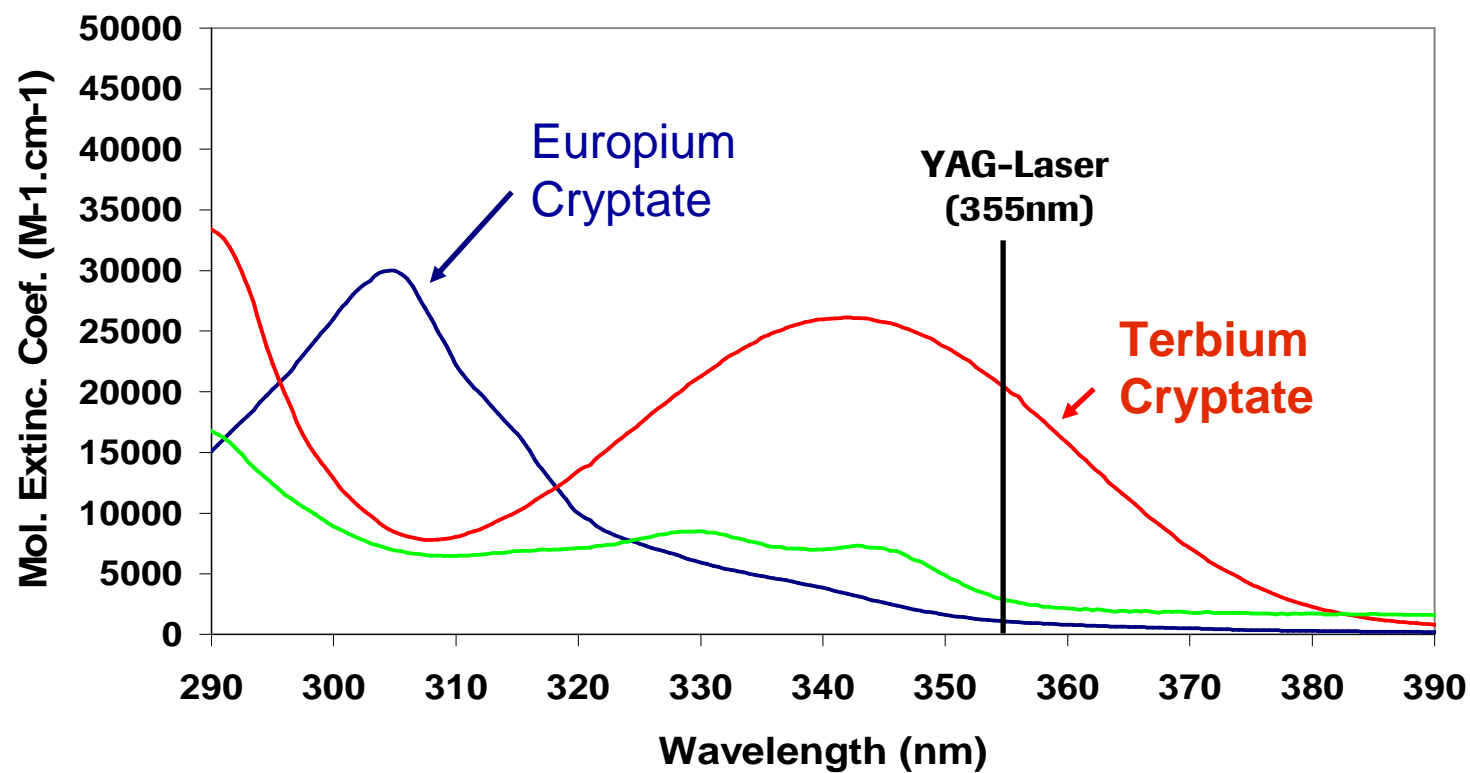
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**First Results with the Terbium Kit**

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**Summary and Conclusion**

# Excitation Spectrum Europium and Terbium





# plate::explorer system and plate::vision reader



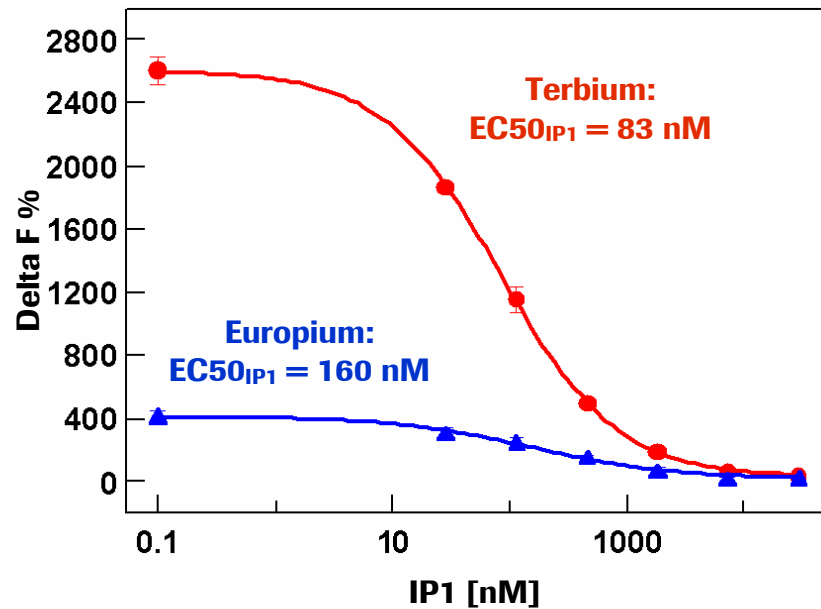
- **Co-development Carl Zeiss Jena and F. Hoffmann-La Roche**
- **Support and Maintenance now by Perkin Elmer**

# Tb and Eu - different plates

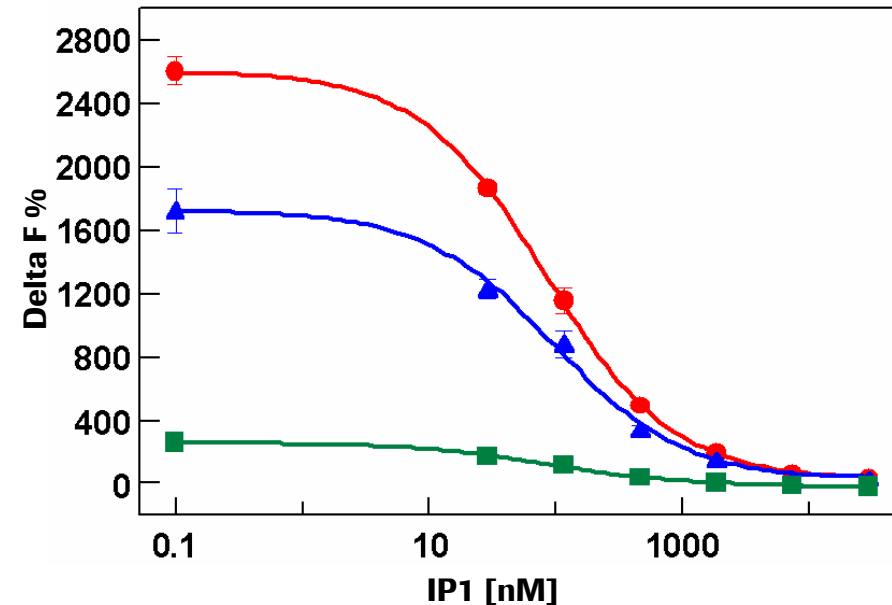


*Standard curves measured on plate::vision reader*

### Tb vs Eu on plate::vision



### Tb in different plates on plate::vision



**Corning black clear bottom (3712): EC<sub>50</sub><sub>IP1</sub> = 83 nM**

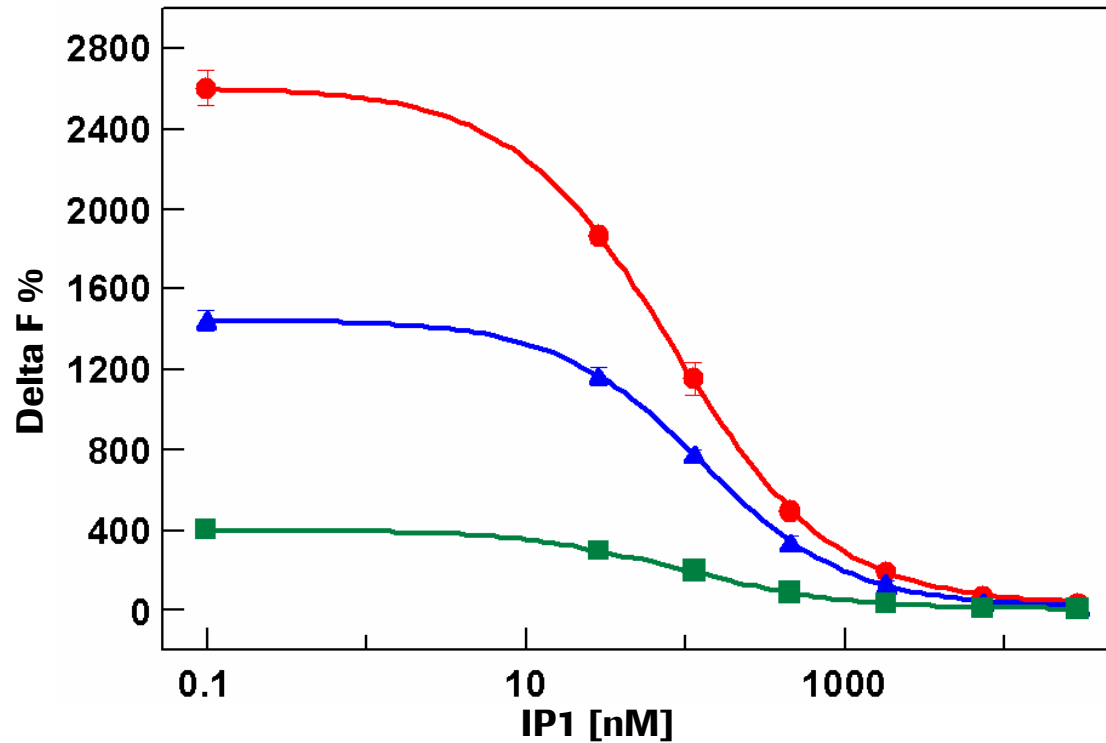
**Corning white clear bottom (3707): EC<sub>50</sub><sub>IP1</sub> = 97 nM**

**Greiner white clear bottom (780098): EC<sub>50</sub><sub>IP1</sub> = 98 nM**

# Different Readers



## Tb standard curves in different readers



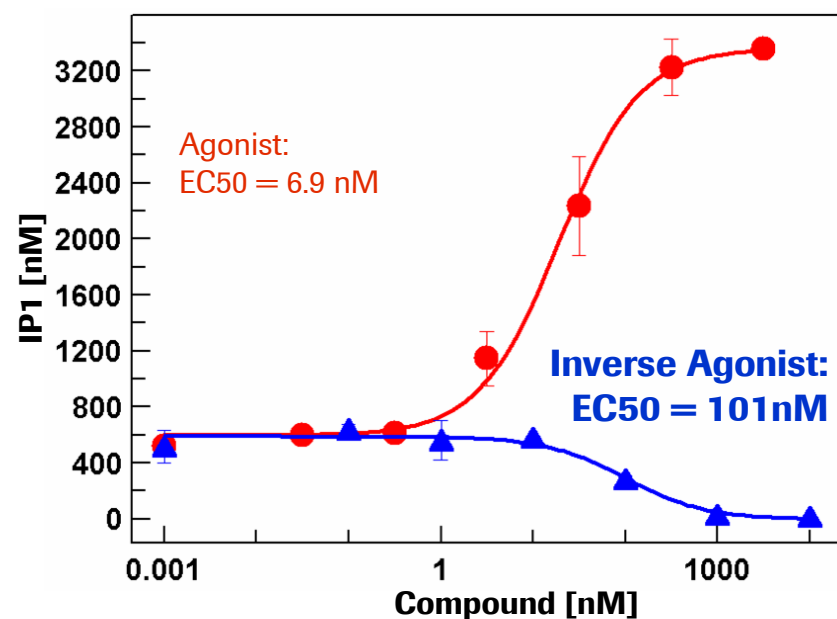
**plate::vision (PerkinElmer)  $EC_{50_{IP1}} = 83 \text{ nM}$**   
**NanoScan (IOM)  $EC_{50_{IP1}} = 130 \text{ nM}$**   
**EnVision (PerkinElmer)  $EC_{50_{IP1}} = 96 \text{ nM}$**

# Cellular Assay with Terbium Assay Kit

## *GPCR2 – inverse agonist project – comparison with Eu*

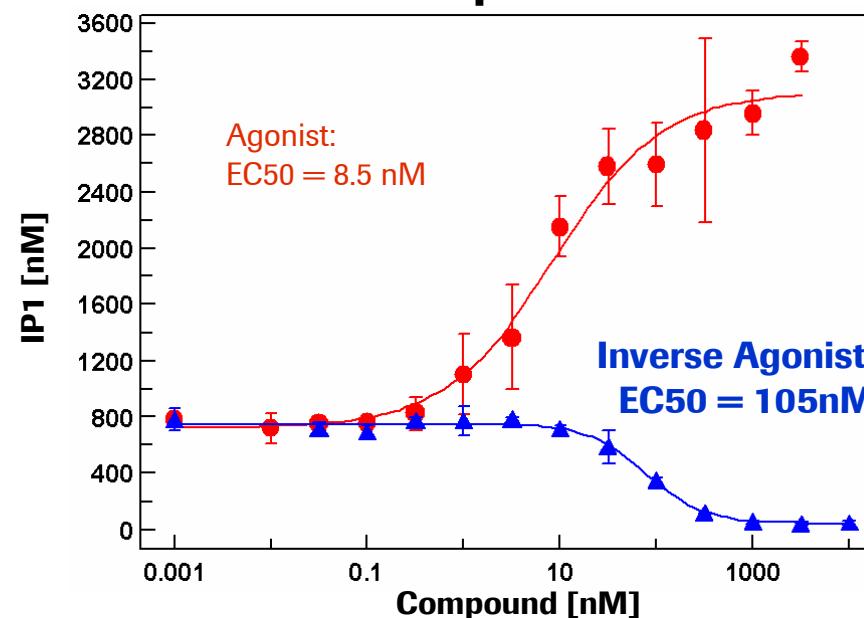
### Optimized inverse agonist conditions

#### Terbium Kit



7'500 cells/well, 4h incubation,  
 Plate: 384 black Corning 3712,  
 Plate::vision reader

#### Europium Kit



7'500 cells/well, 4h incubation,  
 Plate: 384 white Greiner 781080,  
 Discovery reader

# Summary - Conclusion

## Cisbio IP-One Kit

- simple to use and robust
- cell concentration / incubation time needs to be optimized
- GPCR 1: Highly reproducible assay is crucial for optimization of positive modulator
- GPCR 2: Profiling and mode of action: agonist, antagonist, inverse agonist

## Terbium Reagents

- Spectral properties fit very well with plate::vision reader
- 2-4 times more sensitive than Eu-assay
- enables us to use IP-One in primary screening





# Acknowledgments

## **Hoffmann-La Roche, Basel**

- Michel Dietz
- Thilo Enderle
- Doris Roth
- Ramona Schäfer
- Veronique Schirmer

## **Cisbio**

- Stéphane Martinez
- Jean-Luc Tardieu
- Eric Trinquet



*We Innovate Healthcare*