cAMP Hunter™ Follitropin Alfa Bioassay (FSHR)
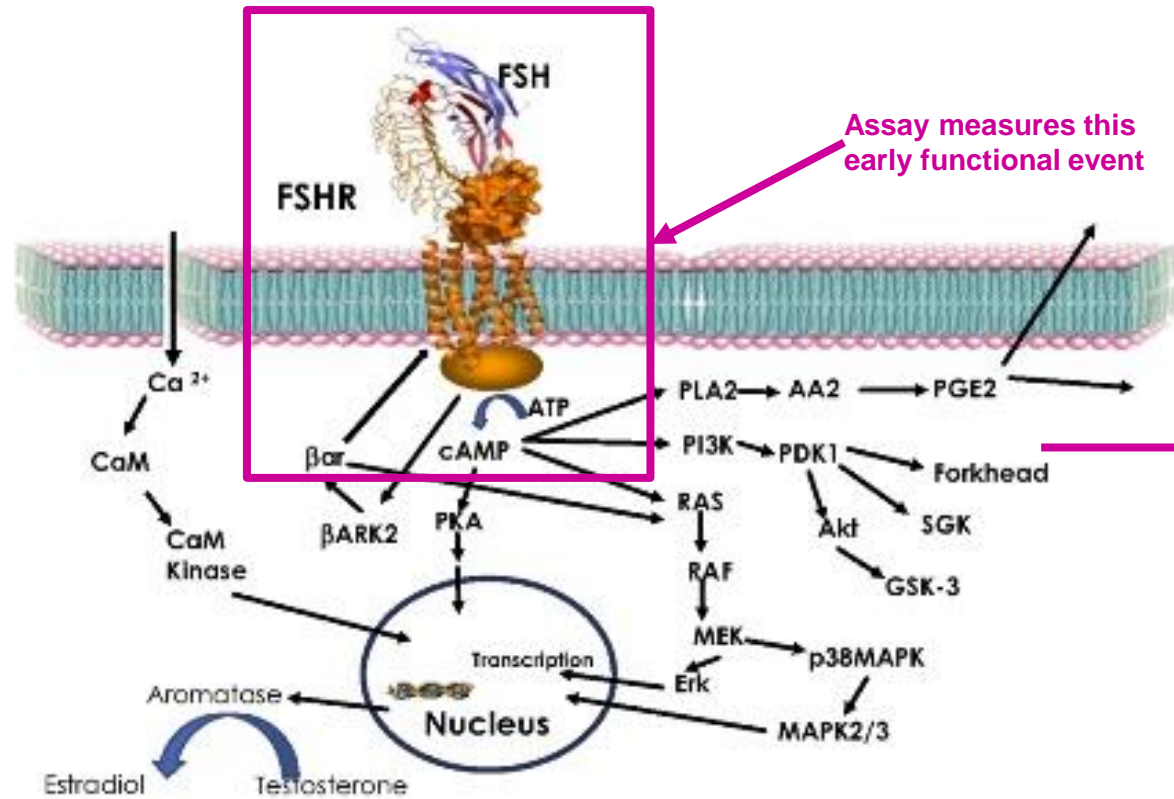
Qualified with Gonal-F®

95-0119Y2-00103 (2-Plate Kit)
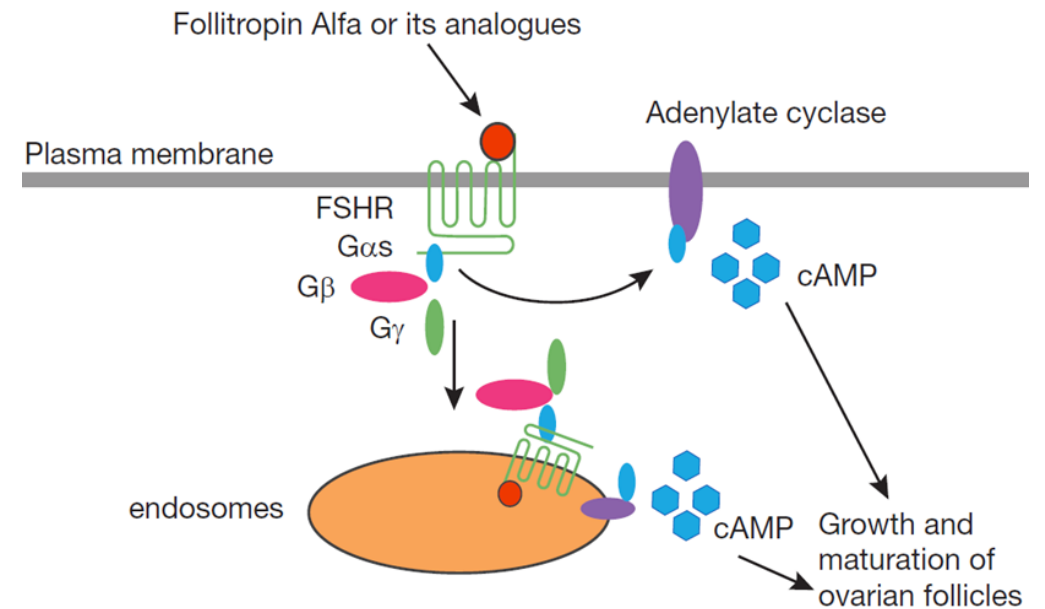
95-0119Y2-00104 (10-Plate Kit)

OUR EXPERTISE
IN YOUR HANDS.

DISCOVER
CONFIDENTLY.

Mechanism of Action

Assay measures this early functional event

Source: Nataraja SG, Yu HN and Palmer SS (2015) Discovery and development of small molecule allosteric modulators of glycoprotein hormone receptors. Front. Endocrinol. 6:142.

Assay Design

Chemiluminescent Detection of cAMP accumulation

cAMP Hunter™ Follitropin Alfa Bioassay Kit

Kit Components

List of Components	95-0119Y2-00103	95-0119Y2-00104
cAMP Hunter CHO-K1 FSHR Bioassay Cells	2 vials	10 vials
cAMP Detection Kit for Bioassays		
cAMP Standard (250 µM) (mL)	0.2	1
cAMP Antibody Reagent (mL)	5	25
cAMP Lysis Buffer (mL)	7.6	38
Substrate Reagent 1 (mL)	2	10
Substrate Reagent 2 (mL)	0.4	2
cAMP Solution D (mL)	10	50
cAMP Solution A (mL)	16	80
Cell Assay Buffer	2 X 50 mL	4 X 50 mL
AssayComplete™ Cell Plating Reagent 2	1 X 100 mL	2 X 100 mL
Ultrapure IBMX	1 vial	1 vial
Control Agonist (FSH)	1 vial	1 vial
96-Well Clear-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

Sample data

Follitropin Alfa Bioassay Kit Qualification

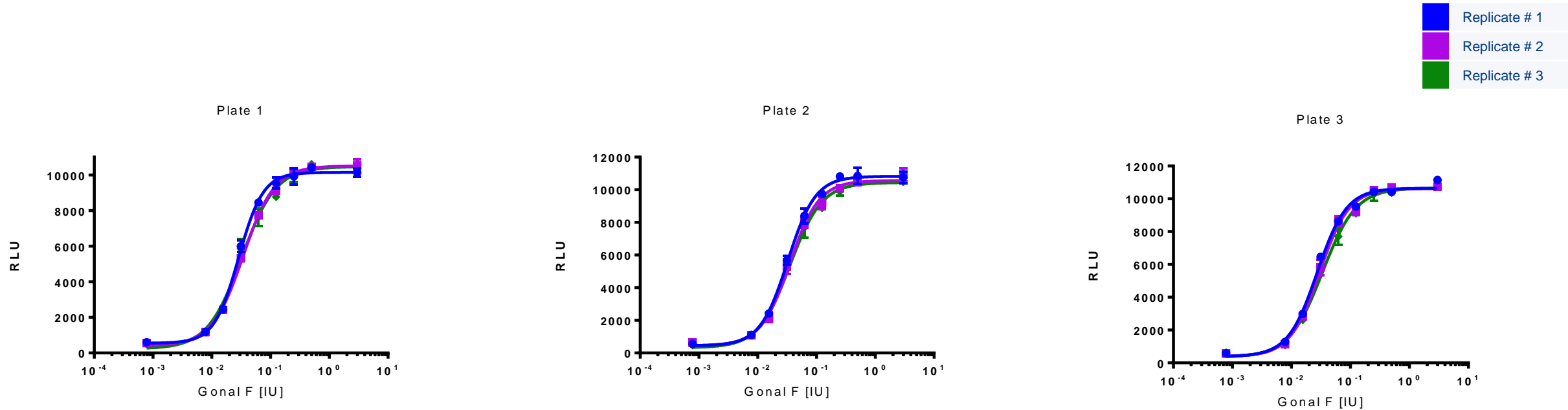# Follitropin Alfa Bioassay Qualification with Gonal-F®
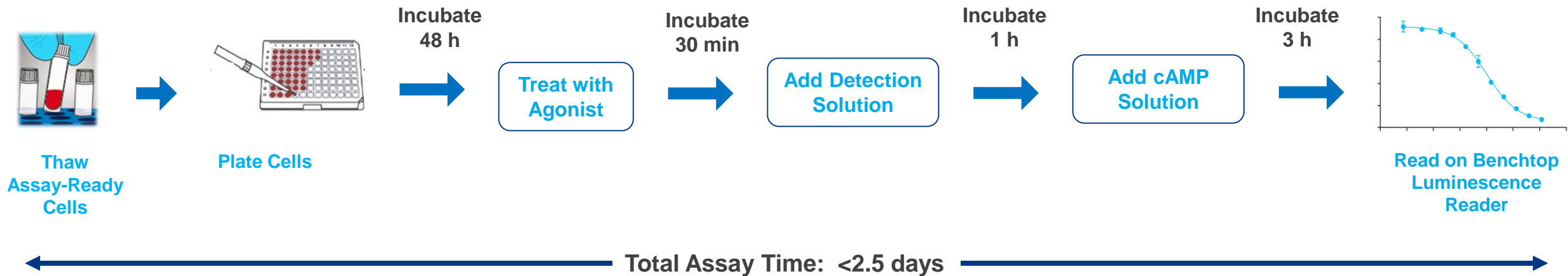
Assay Parameters Assessed

- % CV between eight 11-pt DRCs

- Plate uniformity: EC_{80} and IC_{80} (of drug and stimulus) across entire plate

- Plate-to-Plate variability: 3 plates with 11-pt DRCs run on 3 days

- Slope consistency

- Accuracy, precision and linearity of the assay over a range of 50-150% from two operators
  - Assay developer
  - Assay qualifier

- Parallel line analysis

Follitropin Alfa Bioassay Qualification with Gonal-F®

eurofins | **DiscoverX**
The Eurofins Discovery **PRODUCTS COMPANY**

Plate-to-Plate Variability: 3 Plates with Full-plate DRC - Same Day

- ■ Replicate # 1 (blue)
- ■ Replicate # 2 (purple)
- ■ Replicate # 3 (green)

Plate 1

Plate 2

Plate 3

Parameter	R1	R2	R3
S/B	18.6	18.5	18.0
Hill Slope	2.083	1.624	1.486
EC_{50} (IU)	0.02869	0.03438	0.03225

Parameter	R1	R2	R3
S/B	18.4	19.8	17.9
Hill Slope	1.846	1.742	1.604
EC_{50} (IU)	0.03242	0.03519	0.03546

Parameter	R1	R2	R3
S/B	18.9	19.5	16.8
Hill Slope	1.744	1.728	1.526
EC_{50} (IU)	0.02742	0.02996	0.03346

Follitropin Alfa Bioassay Qualification with Gonal-F®

Assay Robustness

Repeatability and Intermediate Precision (Intra-Day)

Plate	Sample	R2	S/B	EC$_{50}$, IU	Mean EC$_{50}$, IU	%RSD, EC$_{50}$
1	R1	0.996	18.6	0.0287		
	R2	0.998	18.5	0.0344		
	R3	0.990	18	0.0323		
2	R1	0.996	18.4	0.0324	0.0321	8.9
	R2	0.994	19.8	0.0352		
	R3	0.995	17.9	0.0355		
3	R1	0.995	18.9	0.0274		
	R2	0.994	19.5	0.0300		
	R3	0.994	16.8	0.0335		

Intermediate Precision (Inter-Day)

Day	EC$_{50}$, IU	Mean IC$_{50}$, ng/mL	%RSD, IC$_{50}$
1	0.0541		
2	0.0439	0.04	25.4
3	0.0321		

Follitropin Alfa Bioassay Qualification with Gonal-F®

Summary: Accuracy, Precision and Dilutional Linearity (Single Analyst)

Exp #	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1		142.1			
2	150	154.8	154.3	12.0	97.2
3		166.1			
1		121.9			
2	125	132.7	129.0	6.2	96.9
3		132.5			
1		69.5			
2	75	69.5	66.8	4.7	112.3
3		61.4			
1		58			
2	50	44.1	49.6	7.4	100.8
3		46.7			

Note: RP values calculated in GraphPad Prism

Dilutional Linearity

R square | 0.9846
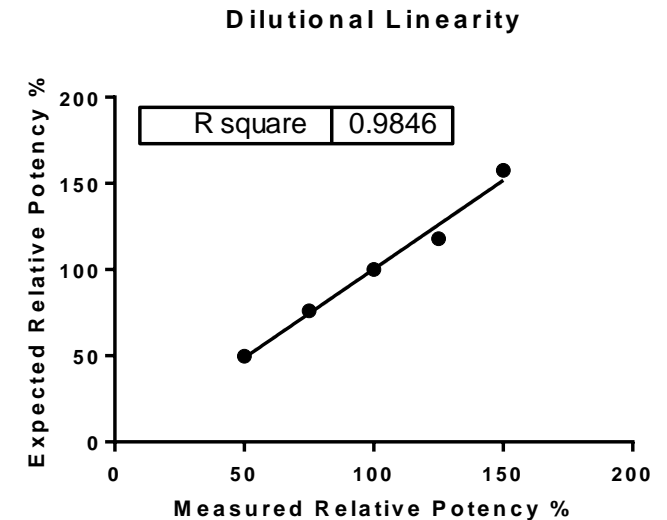
Accuracy: 103.3%
Precision: 6.1%

Follitropin Alfa Bioassay Qualification with Gonal-F®

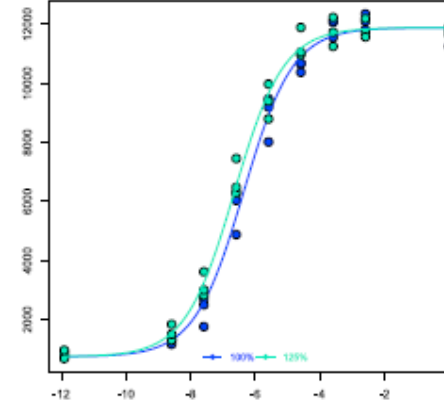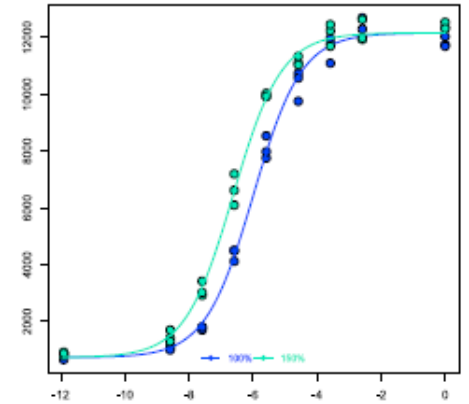*Relative Potency: Parallelism and Potency Estimation (PLA)*
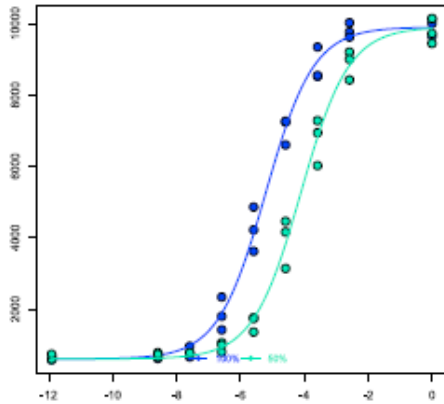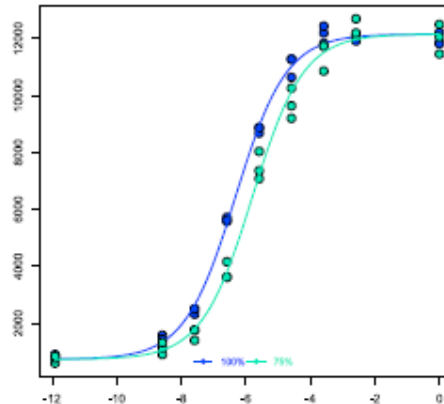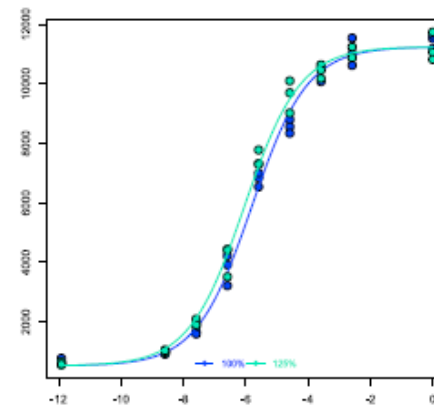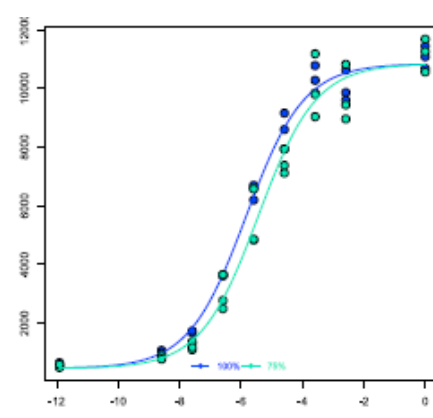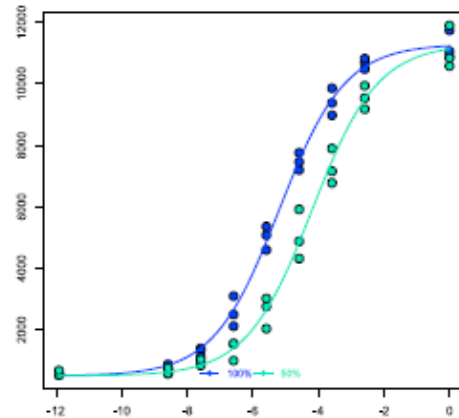
Reference — blue
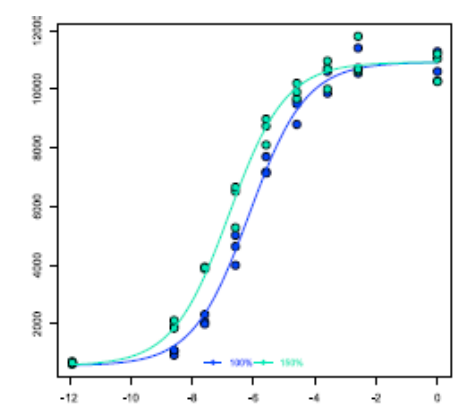**Sample** — green

Note: RP values calculated in PLA 3.0

Benefits for "Ready-to-Use" Bioassay Kits

Functional response based on drug MOA

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

Readily Implement in QC with Optimized kit

- Frozen ready-to-assay cells

- Bioassay Detection Reagents

- Cell Plating Reagent

- Dilution Buffer

- Control Agonist

- Tissue Culture-Treated Plates

For More Info, Questions or Technical Support

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Cell-Based Bioassays for Biologics

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