



cAMP Hunter™ Tirzepatide (GLP-1 RA) Bioassay: Qualification Data

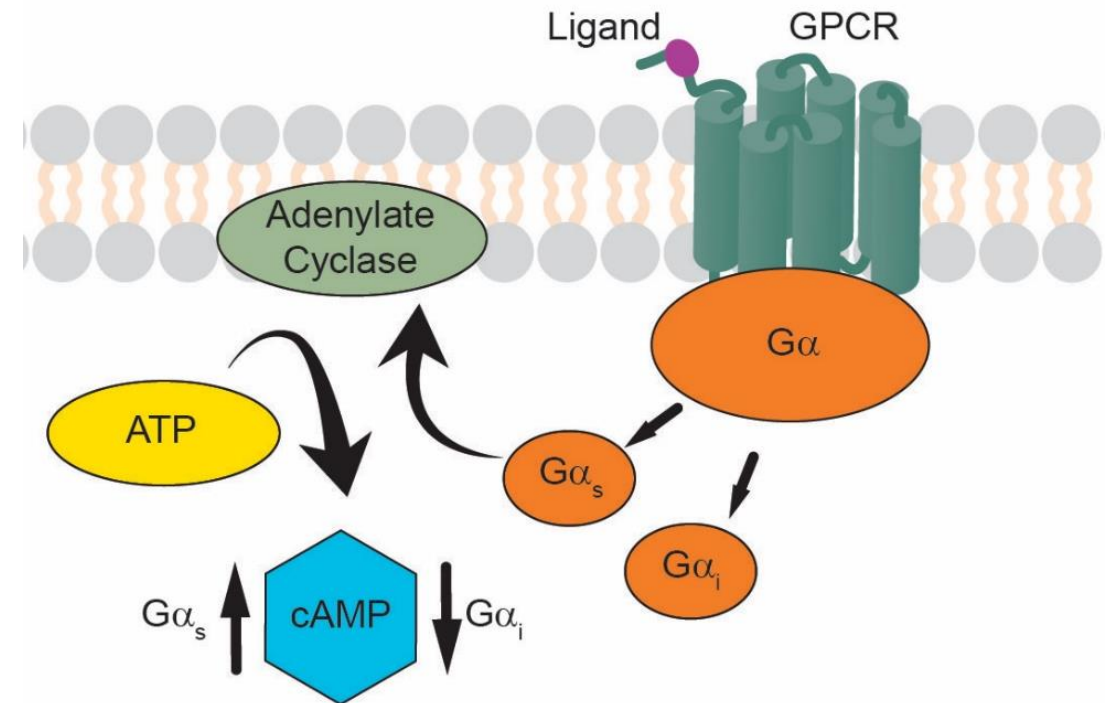
Qualified with Tirzepatide

95-0062Y2-00198- 2-Plate Kit

95-0062Y2-00199- 10 Plate Kit

95-0062Y2-00200- 10 Plate Kit No Control

Background Info: Tirzepatide (marketed as Mounjaro™) is an imbalanced dual GIP and GLP-1 receptor agonist, favoring activation of GIPR over GLP-1R. The cAMP Hunter™ Tirzepatide Bioassay Kit (GLP1R) measures relative potency of Tirzepatide by quantifying cAMP production in response to activation of GLP1R.



Mounjaro is a trademark of Eli Lilly and Company

Bioassay Kit Components

List of Components	95-0062Y2-00198 (2-Plate Kit)	95-0062Y2-00199 (10-Plate Kit)	95-0062Y2-00200 (10 plate Kit without control)
cAMP Hunter CHO-K1 GLP1R Bioassay Cells (3.75 x 10 ⁶ cells in 0.2 mL per vial)	2 Vials	10 Vials	10 Vials
AssayComplete™ Cell Plating 2 Reagent (CP2) (100 mL per bottle)	1 x 100 mL	2 x 100 mL	2 x 100 mL
AssayComplete Cell Assay Buffer (50 mL per bottle)	1 x 50 mL	2 x 50 mL	2 x 50 mL
AssayComplete Protein Dilution Buffer B2(PDB- B2) (50 mL per bottle)	1 x 50 mL	2 x 50 mL	2 x 50 mL
Control Agonist (Human Exendin-4)	1 x 500 µg	1 x 500 µg	N/A*
cAMP Detection Kit for Bioassays			
cAMP Standard (250 µM) (Vial)	1 x 0.2 mL	1 x 1 mL	1 x 1 mL
cAMP Antibody Reagent (Bottle)	1 x 5 mL	1 x 25 mL	1 x 25 mL
cAMP Lysis Buffer (Bottle)	1 x 7.6 mL	1 x 38 mL	1 x 38 mL
Substrate Reagent 1 (Bottle)	1 x 2 mL	1 x 10 mL	1 x 10 mL
Substrate Reagent 2 (Bottle)	1 x 0.4 mL	1 x 2 mL	1 x 2 mL
cAMP Solution D (Bottle)	1 x 10 mL	1 x 50 mL	1 x 50 mL
cAMP Solution A (Bottle)	1 x 16 mL	1 x 80 mL	1 x 80 mL
96-Well White, Clear-Bottom, TC-Treated, Sterile Plates with Lid	2 Plates	10 Plates	10 Plates

*Note: For 95-0062Y2-00200 control not provided in the kit, would need to be obtained separately if needed

Sample data

Tirzepatide Bioassay Qualification

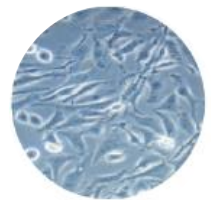
Bioassay Workflow

Simple, Homogenous and Rapid Protocol

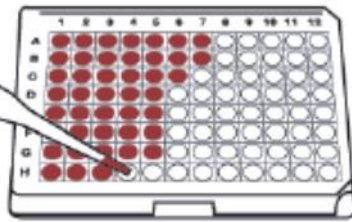
Plate Read-to-Use Cells

Treat with Agonist/ Molecule

Read Luminescence



24 h



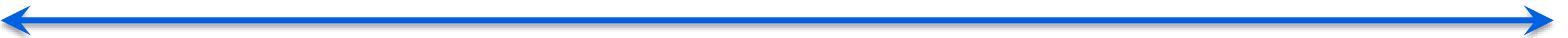
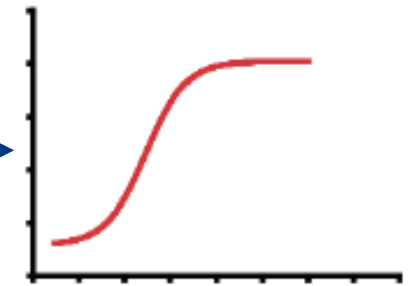
30 min

Add Detection Solution

1 h

Add cAMP Solution

3 h



Dilution Scheme

Dilution Factor	Concentration, g/mL
	8.0E-09
2.5	3.2E-09
2.5	1.3E-09
2.5	5.1E-10
1.5	3.4E-10
1.5	2.3E-10
1.5	1.5E-10
2	7.6E-11
2	3.8E-11
2.5	1.5E-11
2.5	6.1E-12

Representative Dose Curve (100% NC)

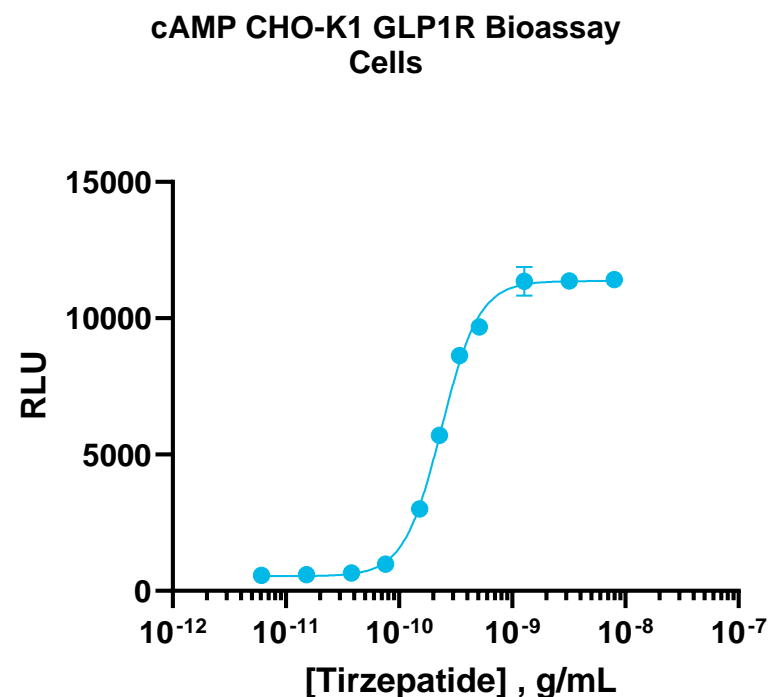
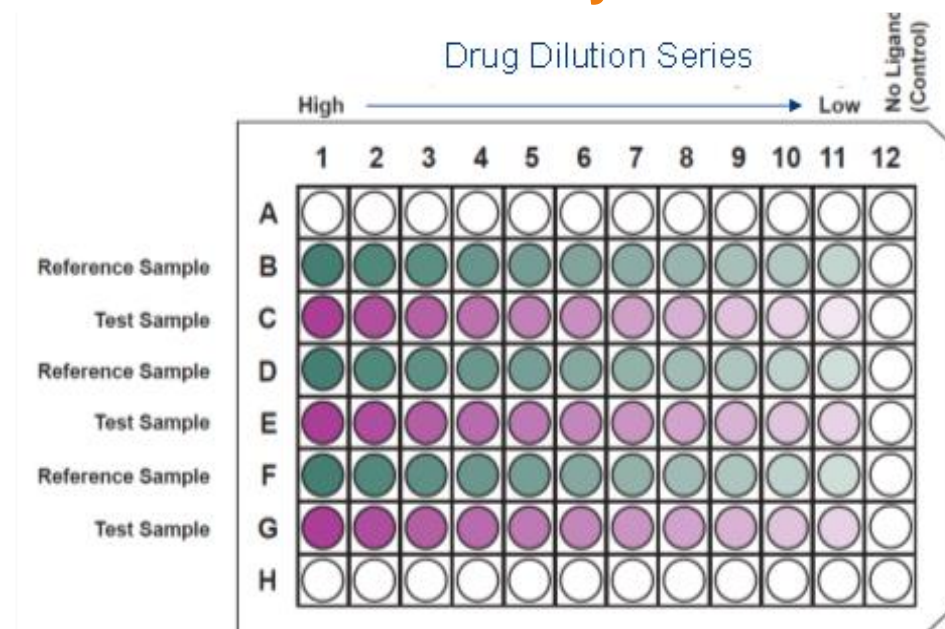


Plate Layout



Day	Nominal Concentrations	Analyst
1	100% x 6 (repeatability)	1
2	150%, 125%, 75%, 50%	1
2/3	150%, 100%, 50%	2
3	150%, 125%, 75%, 50%	1
4	125%, 75%	2
4	150%, 125%, 75%, 50%	1
5	150%, 125%, 75%, 50%	1
6	150%, 125%, 75%, 50%	2
7	Specificity Sample	1 or 2

- Evaluated 5 nominal concentrations (NC) over a range of 50%-150%
 - (n=6 for each NC)
- Repeatability: 6 runs of 100% NC by single analyst
- Intermediate precision incorporates:
 - 2 analysts
 - Multiple days
 - 4 NC
 - 2 lots of bioassay cells
- 100% success rate (e.g. no plates failed system and sample suitability criteria)- see next slide for SST

A. Curve shape: at least 2 data points in the upper and lower asymptotes, and at least 3 data points in the linear portion of the curve

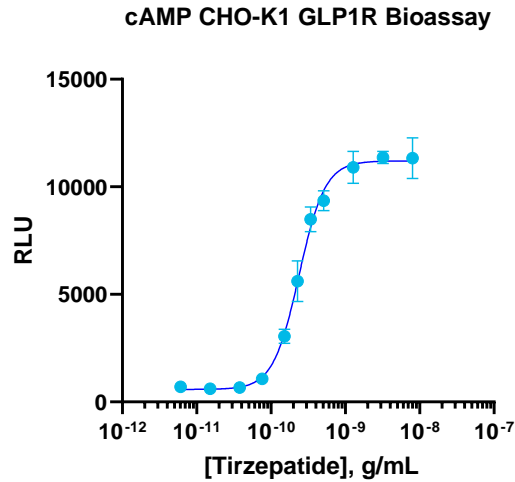
B. Replicate precision: The % CV between replicates of reference standard and test sample wells must be $\leq 25\%$.

C. Signal to Background: The signal to background (upper asymptote / lower asymptote; A/D) ratio of the unconstrained reference standard curve must be ≥ 4 .

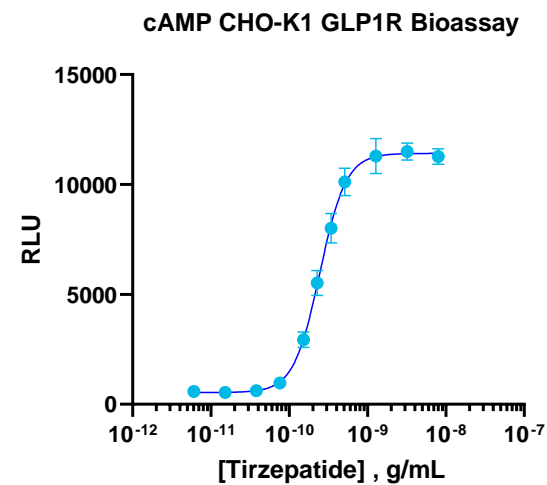
D. Parallelism: Sample must pass F-Test for parallelism in PLA 3.0

Day-to-Day Repeatability with Tirzepatide (Single Analyst)

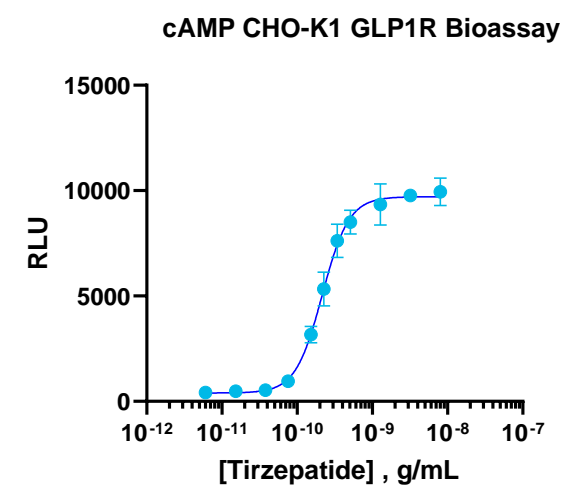
Day 1



Day 2



Day 3



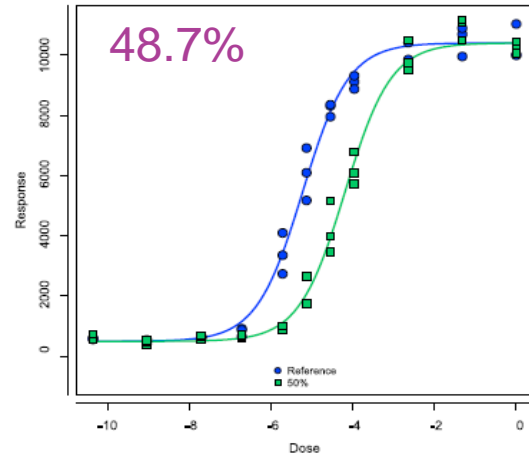
	Day 1	Day 2	Day 3	% RSD
S/B	19.2	21.3	24.4	12.09%
EC ₅₀ , pg/mL	238.4	245.8	215.6	6.74%

Note: consistent data generated with two different bioassay lots

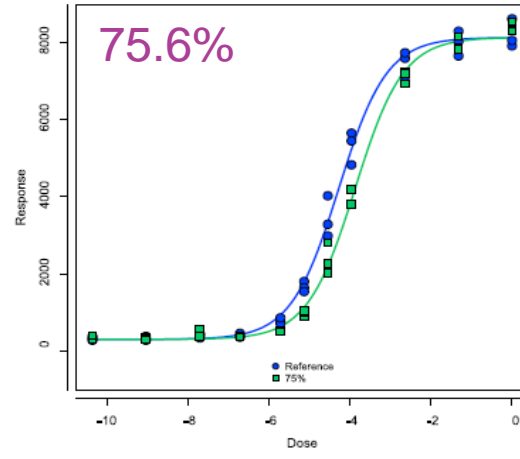
Representative Relative Potency Data with Tirzepatide (4 Nominal Concentrations: Analyst 1 vs Analyst 2)

Analyst 1

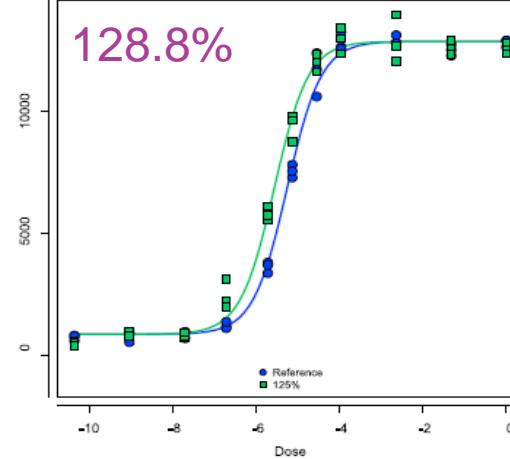
50%



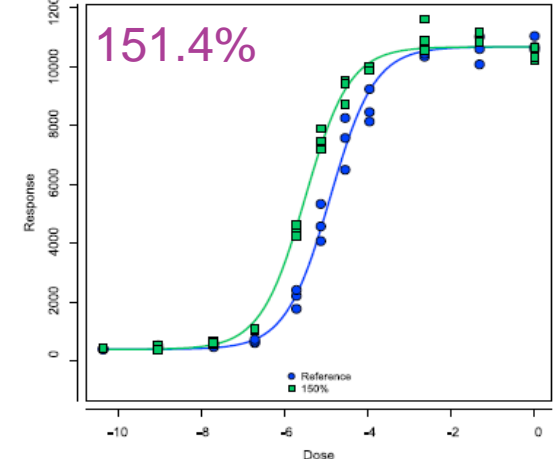
75%



125%

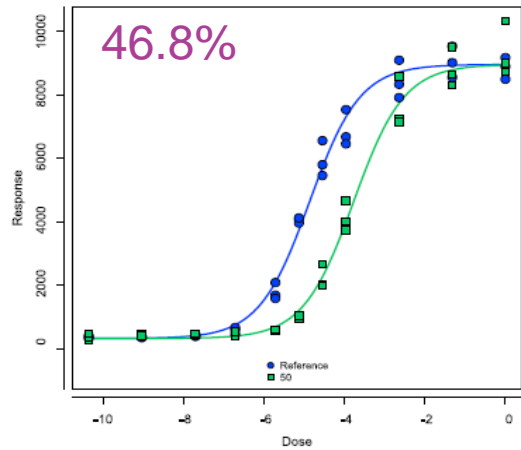


150%

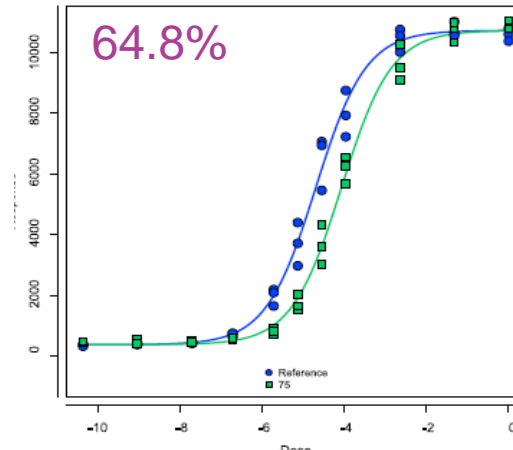


Analyst 2

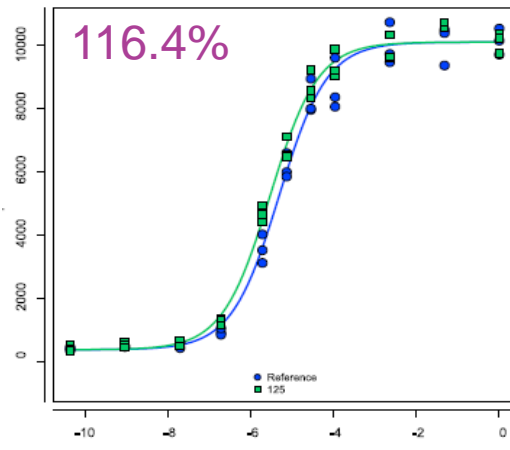
46.8%



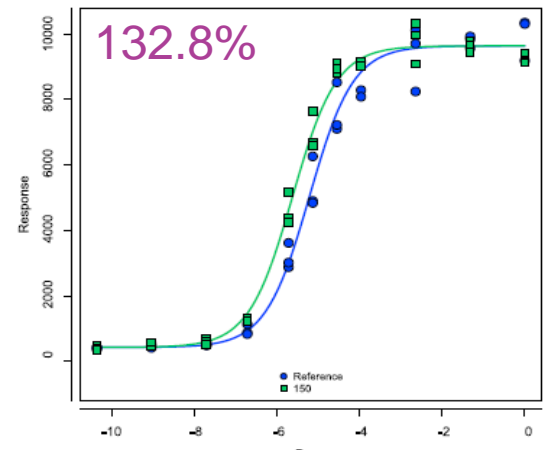
64.8%



116.4%



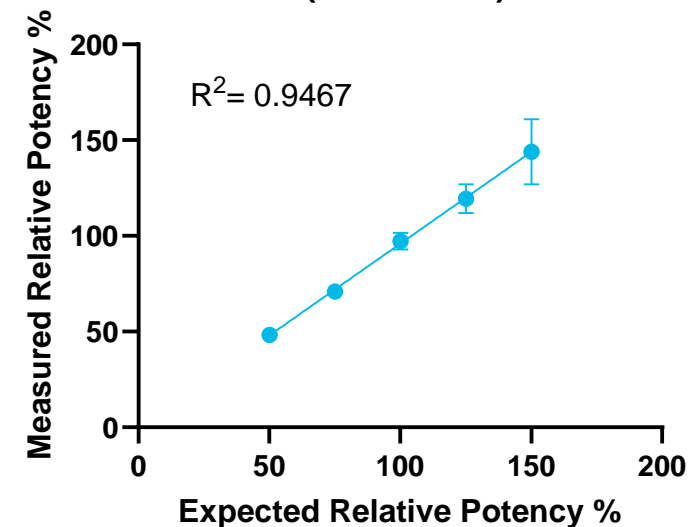
132.8%



Tirzepatide Bioassay Qualification Data

Nominal RP, %	Bioassay Cell Lot	Analyst	Observed RP, %	Average RP, %	RSD, %	Average Recovery, %
150	A	1	174.1	143.9	11.8	95.9
		1	151.4			
		1	127.2			
		1	143.3			
		2	134.7			
	B	2	132.8			
125	A	1	123.8	119.4	6.3	95.5
		1	112.6			
		1	125.8			
		1	128.0			
		2	109.9			
	B	2	116.4			
100	A	1	101.8	97.3	4.5	97.3
		1	95.7			
		1	95.3			
		1	99.2			
		1	101.4			
		1	90.5			
75	A	1	75.6	71.0	5.3	94.7
		1	70.9			
		1	72.6			
		1	73.2			
		2	68.8			
	B	2	64.9			
50	A	1	50.4	48.3	4.4	96.5
		1	48.7			
		1	50.7			
		1	47.9			
		2	45.1			
		2	46.8			
	B	2	46.8			

Tirzepatide Bioassay Dilutional Linearity (50%-150%)



Parameter	Value	Specification
Accuracy (Average % Recovery)	95.2%	100% +/- 20%
Repeatability (100% NC)	4.5%	≤20%
Intermediate Precision	≤11.8%	≤20%
Linearity (R ²)	0.9467	N/A

Excellent accuracy, repeatability, intermediate precision, and dilutional linearity

Functional response based on drug MOA

Verified and Qualified with research grade surrogate of innovator drug

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

Readily Implement with Optimized kit

- Frozen ready-to-assay cells
- Bioassay Detection Reagents
- Cell Plating Reagent
- Dilution Buffer
- Control Agonist
- Tissue Culture-Treated Plates



Web:

[Cell-Based Bioassays for Biologics](#)

Technical Support

discoverx.com/support/