



## cAMP Hunter™ Tirzepatide (GIP RA) Bioassay Kit: Qualification Data

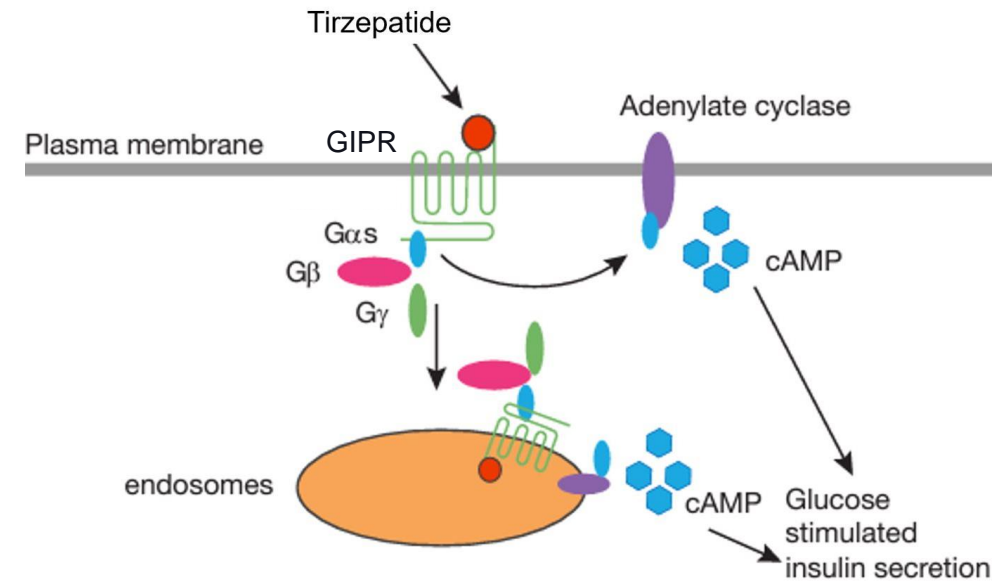
### Qualified with Tirzepatide

- 95-0146Y2-00207: 2-Plate Kit
- 95-0146Y2-00208: 10-Plate Kit
- 95-0146Y2-00209: 10-Plate Kit (No Control)

Background Information: The cAMP Hunter™ Tirzepatide (GIP RA) Bioassay Kit provides an easy-to-use cell-based assay to measure drug potency and detect neutralizing antibodies.

Tirzepatide (LY3298176) is an imbalanced dual Gastric Inhibitory Polypeptide (GIP) and Glucagon-like peptide 1 (GLP-1) receptor agonist, favoring activation of GIPR over GLP-1R. This bioassay assesses ligand mediated activation of GIPR activity via detection of cyclic AMP (cAMP).

This assay has been optimized and qualified with Tirzepatide (not supplied in the bioassay kit). Bioassay kits are a convenient, ready-to-use format which contains all materials needed to run the assay, including single-use vials of cryopreserved cells, cell plating media, control agonist, detection reagent, and assay plates. A 10-Plate cAMP Hunter™ Tirzepatide (GIP RA) Bioassay Kit is also offered without the positive control agonist but contains all other components listed above to run the assay.



Ligand-mediated activation of GIPR by Tirzepatide stimulates adenylate cyclase, which in turn enables the production of cAMP, which is measured with cAMP Detection Kit.

# cAMP Hunter™ Tirzepatide (GIP RA) Bioassay Kit Components

List of Components	95-0146Y2-00207 (2-Plate Kit)	95-0146Y2-00208 (10-Plate Kit)	95-0146Y2-00209 (10 plate Kit without control)
cAMP Hunter CHO-K1 GIPR Bioassay Cells (2.2 x 10 <sup>6</sup> 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
Control Agonist (GIP), 500 µg	1 x 500 µg	1 x 500 µg	N/A*
<b>cAMP Detection Kit for Bioassays</b>			
cAMP Standard (250 µM) (Bottle)	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, Flat-bottom Poly-D-Lysine-coated, Sterile Plates with Lid	2 Plates	10 Plates	10 Plates



Representative Data

# cAMP Hunter™ Tirzepatide (GIP RA) Bioassay Qualification

# Bioassay Workflow

*Simple, Homogenous and Rapid Protocol*

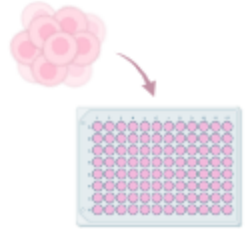
Plate Read-to-Use Cells

Treat with Agonist/ Molecule

Add Detection Solution

Add cAMP Solution

Read Luminescence



24 h



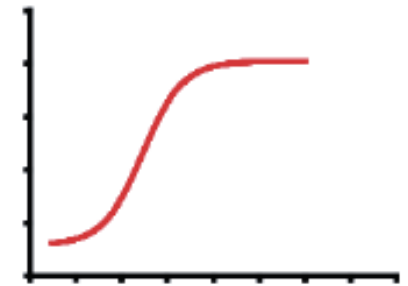
30 min



1 h



2 h

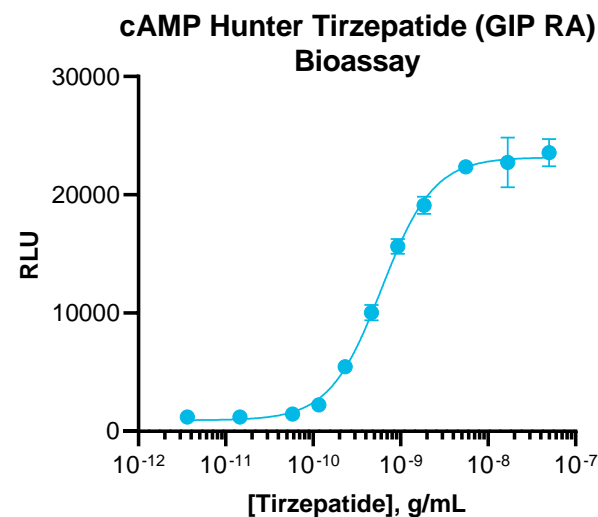


# Optimized Dose Curve and Plate Layout For Qualification of the cAMP Hunter™ Tirzepatide (GIP RA) Bioassay

## Dilution Scheme

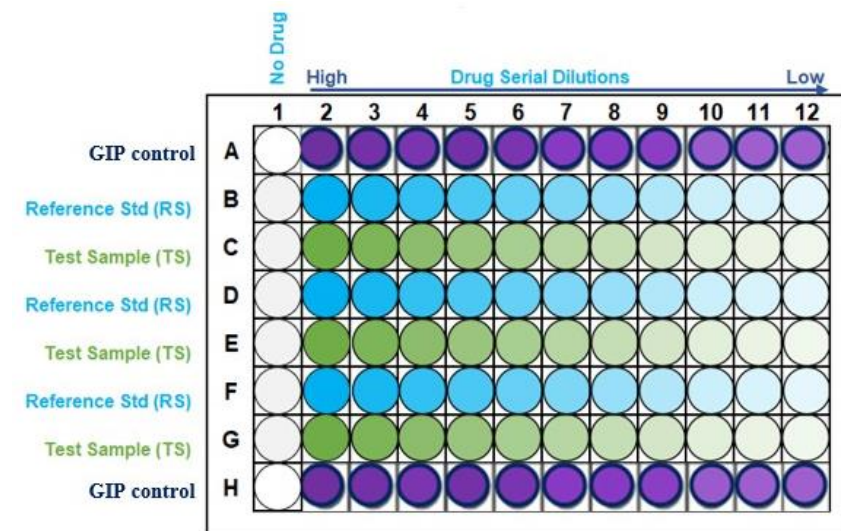
For Tirzepatide	
Dilution Factor	Concentration, ng/mL
	50
3	16.6667
3	5.5556
3	1.8519
2	0.9259
2	0.4630
2	0.2315
2	0.1157
2	0.0579
4	0.0145
4	0.0036

## Representative Dose Curve (100% NC)



S/B	EC <sub>50</sub> , pg/mL
19.3	603.9

## Plate Layout



# Example of Plate Uniformity with EC<sub>80</sub> of Tirzepatide in cAMP Hunter™ Tirzepatide (GIP RA) Bioassay

## % Difference from plate mean

	1	2	3	4	5	6	7	8	9	10	11	12
A	-10%	-11%	-10%	0%	0%	15%	-1%	0%	-7%	-1%	0%	-2%
B	-10%	2%	-10%	-5%	-6%	-2%	-11%	-9%	-14%	-7%	-11%	0%
C	-3%	8%	9%	6%	13%	16%	12%	8%	8%	-4%	-4%	-1%
D	-2%	6%	2%	6%	16%	10%	8%	3%	1%	-1%	-9%	-14%
E	-3%	1%	3%	12%	12%	17%	9%	1%	3%	-2%	-10%	-18%
F	-11%	3%	0%	7%	1%	-3%	2%	1%	-2%	2%	-3%	-18%
G	-2%	14%	9%	9%	3%	11%	-1%	3%	-6%	-12%	6%	-7%
H	-9%	2%	-7%	7%	-10%	0%	1%	6%	-1%	-9%	-9%	1%

Parameter	Day 1
%CV (across plate)	8.0%
Inter-row % CV	3.9%
Inter-column % CV	4.9%

**Acceptance Criteria (to use entire plate):** % CV across plate: ≤15%; %CV for inter-row and inter-column: ≤10%.

*Note: Bioassay optimized to use on entire plate.*

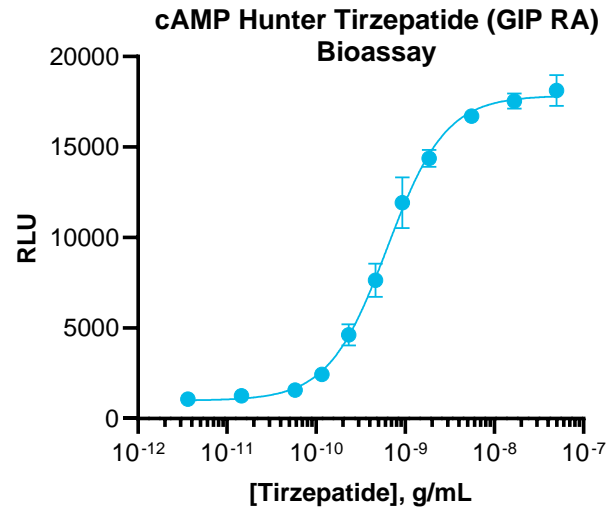
Day	Nominal Concentrations	Analyst
1	100% x 6 (repeatability)	1
2	200%, 140%, 70%, 50%	1
2/3	200%, 100%, 50%	2
3	200%, 140%, 70%, 50%	1
4	140%, 70%	2
4	200%, 140%, 70%, 50%	1
5	200%, 140%, 70%, 50%	1
6	200%, 140%, 70%, 50%	2

- Evaluated plate uniformity for positional variability and edge effects
- Evaluated 5 nominal concentrations (NC) over a range of 50%-200% (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

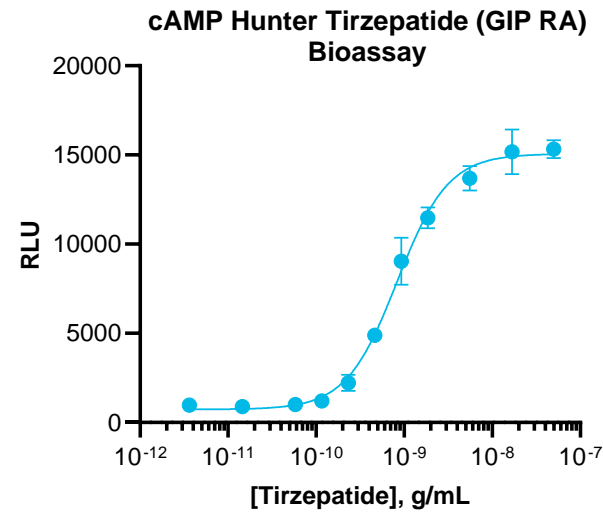


# Day-to-Day Repeatability (Single Analyst) in the cAMP Hunter™ Tirzepatide (GIP RA) Bioassay

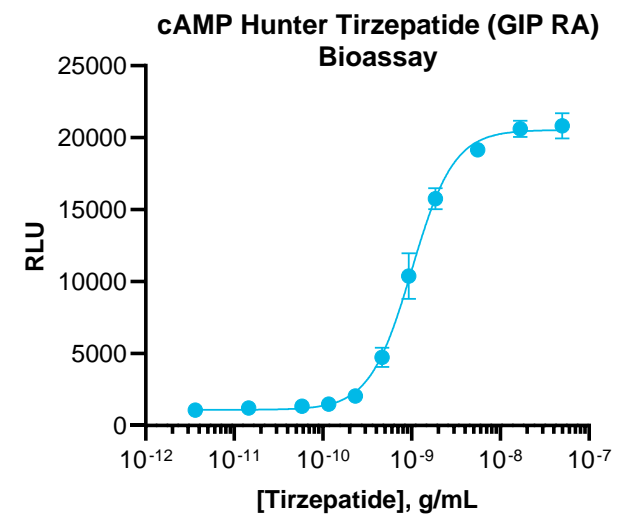
Day 1 (Lot 1)



Day 2 (Lot 1)



Day 3 (Lot 2)



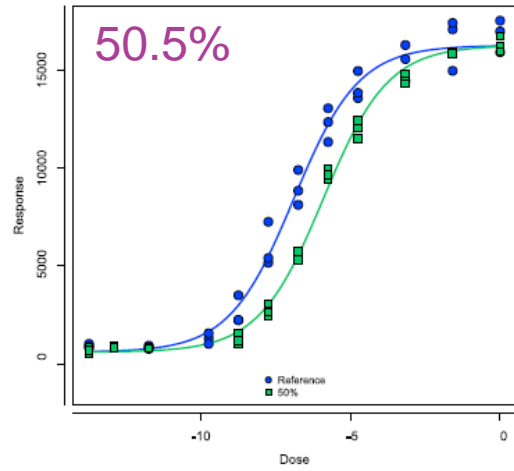
Parameter	Day 1	Day 2	Day 3	% RSD
S/B	18.0	20.6	18.8	6.9%
EC <sub>50</sub> , pg/mL	625.1	829.6	1001	22.9%

*Note: consistent data generated over multiple days and with two different bioassay lots*

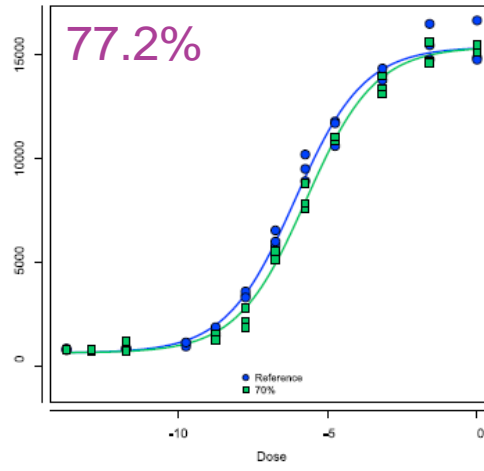
# Representative Relative Potency Data (4 Nominal Concentrations over a range from 50% to 200%): Analyst 1 vs Analyst 2

Analyst 1

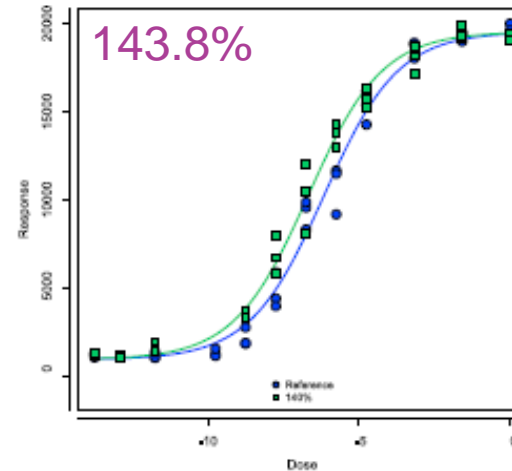
50%



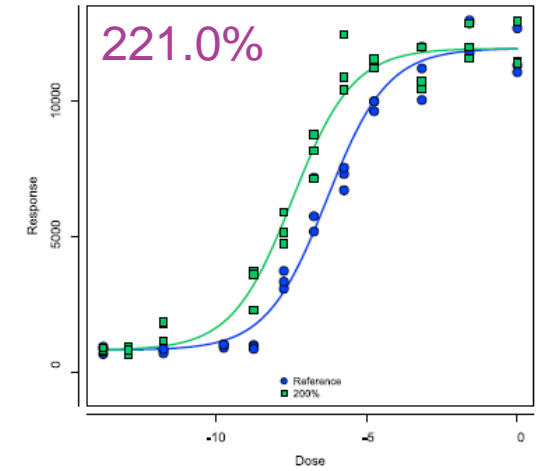
70%



140%

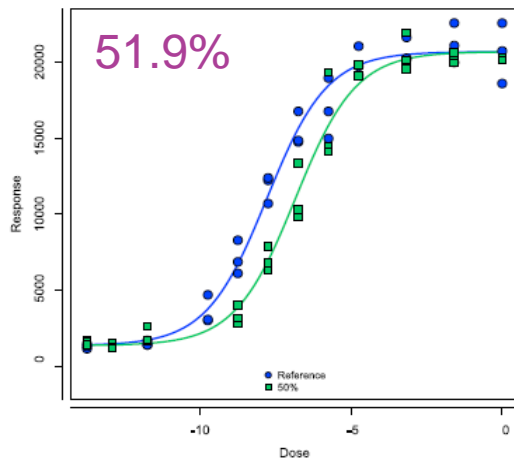


200%

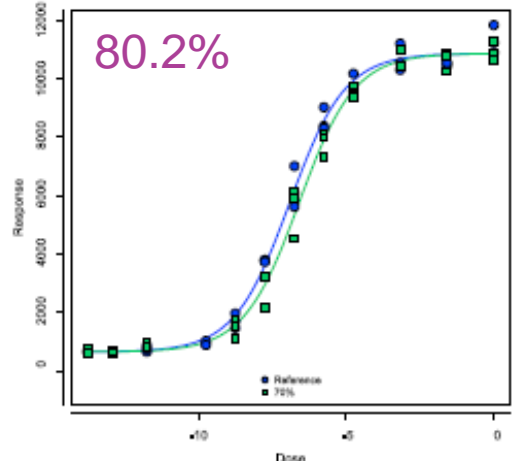


Analyst 2

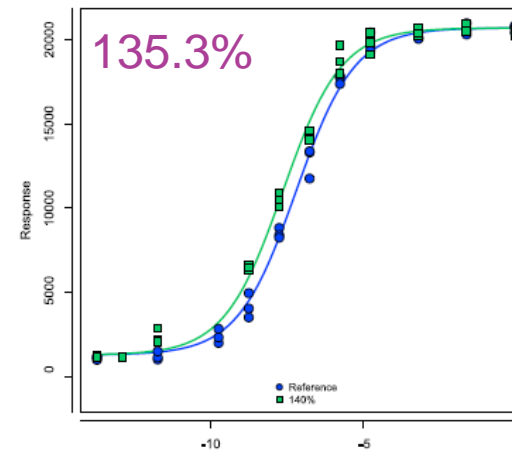
51.9%



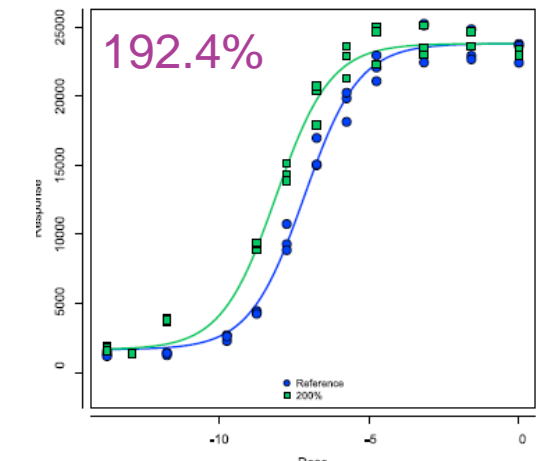
80.2%



135.3%



192.4%

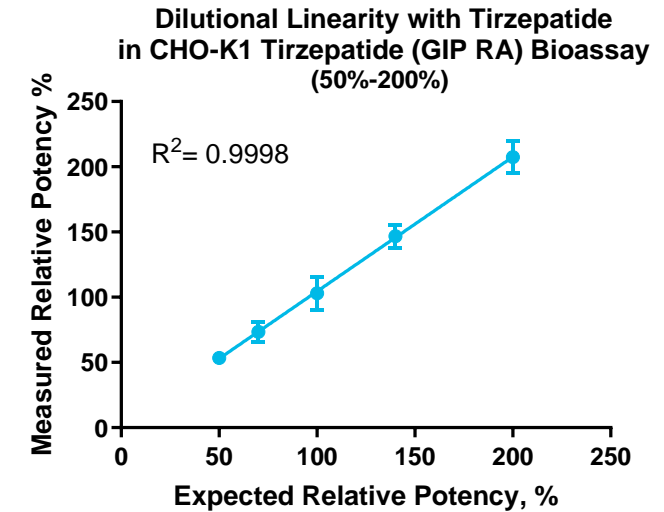


# Assessment of Repeatability with 100% NC (Single Analyst)

Expected RP, %	Exp # / Plate #	Analyst	Measured RP, %	Potency Range, 95% CI, %	Mean RP, %	% RSD
100	1/1	1	106.6	0.93487 - 1.21599	103.1	12.4%
	1/2	1	111.1	0.93401 - 1.32208		
	1/3	1	96.1	0.82442 - 1.12015		
	1/4	1	120	1.04394 - 1.37835		
	1/5	1	101.5	0.88839 - 1.15890		
	1/6	1	83	0.73412 - 0.93828		

# Summary of Tirzepatide Bioassay Qualification Data: Accuracy, Intermediate Precision and Dilutional Linearity

Nominal RP, %	Exp#	Bioassay Cell Lot	Analyst	Observed RP, %	Average RP, %	RSD, %	Average Recovery, %
200	1	A	1	222.8	207.4	6.1	103.7
	2		1	221.0			
	3		1	196.3			
	4		1	202.5			
	5		2	192.4			
	6	B	2	209.4			
140	1	A	1	153.3	146.8	6.0	104.9
	2		1	143.9			
	3		1	159.7			
	4		1	140.9			
	5		2	135.3			
	6	B	2	147.4			
100	1	A	1	106.6	103.8	11.4	103.8
	2		1	111.1			
	3		1	96.1			
	4		1	120			
	5		1	101.5			
	6		1	83			
	7	B	2	108.5			
70	1	A	1	78.7	73.5	10.3	105.0
	2		1	77.3			
	3		2	59.4			
	4		2	73.1			
	5		1	80.2			
	6	B	1	72.3			
50	1	A	1	57.7	53.5	5.7	107.0
	2		1	50.5			
	3		1	56.1			
	4		1	54.1			
	5		2	51.9			
	6	B	2	50.5			



Parameter	Value	Specification
Accuracy (Average % Recovery)	104.9%	100% +/- 20%
Repeatability	12.4%	≤20%
Intermediate Precision	≤11.4%	≤20%
Linearity ( $R^2$ )	0.9998	≥0.95

*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/](https://discoverx.com/support/)