User Manual

cAMP Hunter™ GLP-1 RA Bioassay Kit

For the Measurement of Ligand-Mediated cAMP Accumulation

For Bioassay Kits with control

95-0062Y2-00201: 2-Plate Kit

95-0062Y2-00202: 10-Plate Kit

For Bioassay Kits without control

95-0062Y2-00203: 10-Plate Kit

Document Number 70-448 Rev0

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Important: Please read this entire user manual before proceeding with the assay.

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1. Overview

The cAMP Hunter GLP-1 RA Bioassay Kit provides a robust and highly sensitive functional, cell-based assay to monitor 3'-5'-cyclic adenosine monophosphate (cAMP) production in cells as a result of ligand-mediated G-protein coupled receptor (GPCR) activation. The bioassay kit contains all the materials needed for a complete assay, including cryopreserved ready-to-assay cells, cell plating reagent, dilution buffer, positive control agonist, assay plates, and detection reagents. A 10-Plate cAMP Hunter™ GLP-1 RA Bioassay Kit without control agonist is also offered. This kit contains all other components listed above to run the assay. The ready-to-use cryopreserved cells have been manufactured to ensure assay reproducibility and faster implementation from characterization to lot release. This bioassay has been optimized for a 96-well format but can be adapted to a 384-well format if needed.

2. Assay Principle

Ligand-mediated GPCR stimulation leads to the activation of G-proteins, which trigger downstream signaling pathways by recruiting, activating, or inhibiting cellular enzymes. One such enzyme is adenylate cyclase, which catalyzes the conversion of adenosine triphosphate (ATP) to cyclic AMP (cAMP). Adenylate cyclase is either stimulated or inhibited by the G-protein subunits, Gαs and Gαi, respectively. In the cAMP Hunter GLP-1 RA Bioassay Kit, cells overexpressing Glucagon-like Peptide-1 Receptor (GLP-1R) utilize the natural coupling status of the GPCR to Gas to monitor activation of the receptor. When GLP-1R is activated by agonist for example: Exendin-4, this stimulates adenylate cyclase, which in turn enables production of cAMP. The resulting increase in cellular cAMP levels is measured using a homogenous, gain-of-signal competitive immunoassay based on Enzyme Fragment Complementation (EFC) technology.

The EFC technology uses a β-galactosidase (β-gal) enzyme split into two fragments, the Enzyme Donor (ED) and Enzyme Acceptor (EA). Independently, these fragments have no β-gal enzymatic activity; however, in solution, they rapidly complement to form an active β-gal enzyme. In this assay, cAMP from cell lysates and ED-labeled cAMP (ED-cAMP) compete for anti-cAMP antibody (Ab). Antibody-bound ED-cAMP cannot complement with EA, but unbound ED-cAMP is free to complement with EA and form active β-gal. Any β-gal formed subsequently acts upon the substrate in the detection reagent to produce a chemiluminescent signal. The signal from the assay is directly proportional to the amount of cellular cAMP in the well; i.e., the higher the GLP1R activation, the greater the cAMP levels inside the cells, and the larger the signal produced in the assay.

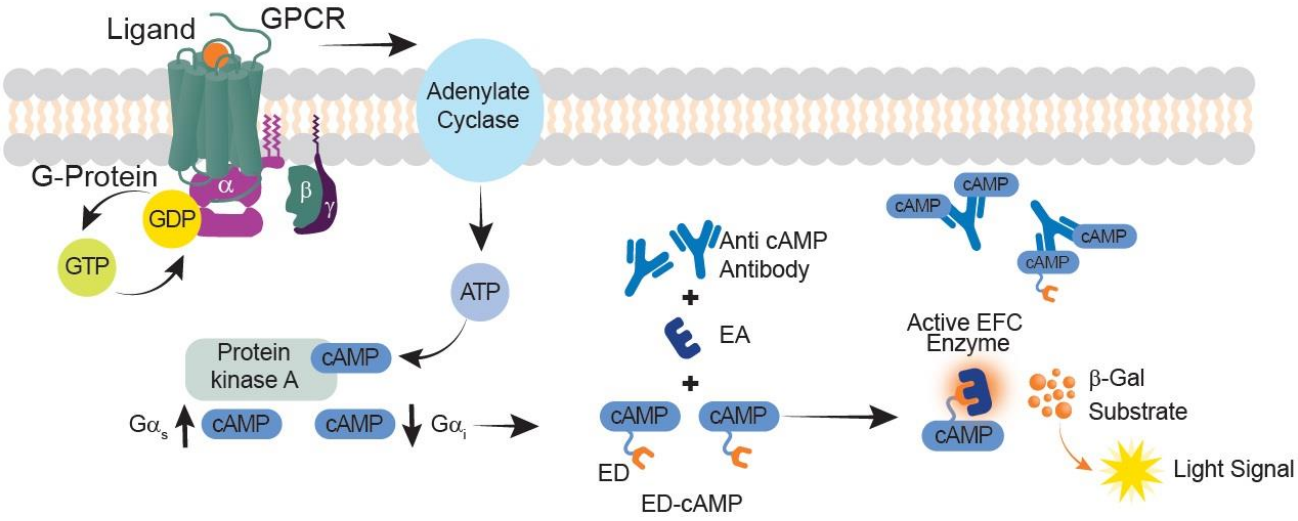


Figure 1. Assay Principle

When cellular cAMP levels are low, ED-labeled cAMP successfully binds with the anti-cAMP-antibody, as there are an insufficient number of cellular cAMP molecules to compete against it. In contrast, when cellular cAMP levels are high, the cellular cAMP molecules bind to the anti-cAMP antibody instead, leaving ED-labeled cAMP relatively free. Upon the addition of the detection reagent containing EA, the ED and EA fragments undergo complementation that successfully forms an active β-galactosidase enzyme that hydrolyzes the substrate and generates a chemiluminescent signal.

3. Materials Provided

List of Components	95-0062Y2-00201 (2-Plate Kit)	95-0062Y2-00202 (10-Plate Kit)	95-0062Y2-00203 (10-plate Kit without control)
cAMP Hunter CHO-K1 GLP1R Bioassay Cells (3.75×10^6 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 (Recombinant Human Exendin-4)	1 x 500 µg	1 x 500 µg	N/A*
cAMP Detection Kit for Bioassays			
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, Clear-Bottom, TC-Treated, Sterile Plates with Lid	2 Plates	10 Plates	10 Plates

***Note**: For 95-0062Y2-00203, a control agonist is not provided in the kit and must be obtained separately if needed. Refer to the additional materials required table for ordering information for the control agonist.

4. Storage Conditions

cAMP Hunter CHO-K1 GLP1R Bioassay Cells

Cells are shipped on dry ice and should arrive in a frozen state. To ensure maximum cell viability, store the vials of bioassay cells in the vapor phase of liquid nitrogen upon receipt. Please contact technical support immediately if the cells received were already thawed.

- Short-term (24 hours or less): Store vials at -80°C immediately upon arrival. (DO NOT store at -80°C for longer than 24 hours).

- Long-term (greater than 24 hours): Vials should ONLY be stored in the vapor phase of liquid nitrogen.

Safety Warning: A face shield, gloves, and lab coat should be worn at all times when handling frozen vials. Use tongs to remove cryovials from liquid nitrogen storage and place the vials immediately on dry ice in a covered container. Wait at least 1 minute for any liquid nitrogen that may be present inside the vial to evaporate. Do not touch the bottom of the vials at any time to avoid inadvertent thawing of the cells.

AssayComplete™ Cell Plating 2 Reagent (CP2)

Upon receipt, store at -20°C. Once thawed, the Cell Plating 2 Reagent can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagent should be aliquoted and stored at -20°C. Once aliquoted, do not freeze-thaw more than two times. Make aliquots suitable for testing one assay plate; for example, 20 mL of reagent per aliquot can be dispensed and stored at -20°C. Ensure that the reagent is equilibrated to room temperature before use in the assay for best performance.

AssayComplete Cell Assay Buffer

Upon receipt, store at -20°C. Once thawed, the Cell Assay Buffer can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagent should be aliquoted and stored at -20°C. Once aliquoted, do not freeze-thaw more than twice. Make aliquots suitable for testing one assay plate; for example, 10 mL of reagent per aliquot should be dispensed and frozen down. This amount may vary depending on your stock sample concentrations and should be adjusted accordingly. Ensure that the reagent is equilibrated to room temperature before use in the assay for best performance.

AssayComplete Protein Dilution Buffer B2 (PDB-B2)

Upon receipt, store at -20°C. Once thawed, the Protein Dilution Buffer B2 can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagent should be aliquoted and stored at -20°C. Once aliquoted, do not freeze-thaw more than twice. Make aliquots suitable for testing one assay plate. For example:10 mL of reagent per aliquot should be dispensed and frozen down. This amount may vary depending on your stock sample concentrations and should be adjusted accordingly. Ensure that the reagent is equilibrated to room temperature before use in the assay for best performance.

Control Agonist (Recombinant Human Exendin-4)

Upon receipt, store at -20°C until ready to use (up to the expiration date listed on the kit’s Certificate of Analysis). To avoid condensation, equilibrate the vial to ambient temperature before opening. Centrifuge the vial prior to opening to maximize recovery and reconstitute using the supplied reconstitution buffer as indicated in this protocol (Page 12). The reconstituted ligand is stable for 12 months at -20 to -80°C if stored in smaller aliquots (avoid multiple freeze-thaw cycles) or 1 week at 2-8°C.

cAMP Detection Kit for Bioassays

Upon receipt, store the kit at -20°C. Once thawed, the kit components can be stored at 4°C for up to 4 weeks. For longer storage (up to the expiration date listed on the kit's Certificate of Analysis), the reagents should be aliquoted and stored at -20°C in opaque containers until needed. Once aliquoted, do not freeze-thaw more than twice. Make aliquots suitable for testing one assay plate. For example:10 mL of reagent per aliquot should be dispensed and frozen down. This amount may vary depending on your

stock sample concentrations and should be adjusted accordingly. Ensure that the reagents are equilibrated to room temperature before use in the assay for best performance.

96-Well Clear Bottom Tissue Culture-Treated Plates

Upon receipt, store at room temperature.

5. Additional Materials Required

The equipment and additional materials below are required to perform these assays but are not included in the assay. Equivalent reagents may be used. All plastics materials should be stored at temperatures specified by suppliers.

Material	Ordering Information
*Positive Control Agonist (Recombinant Human Exendin-4)	DiscoverX, Cat No. 92-1115, or similar
96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plates	DiscoverX, Cat No. 92-0011, or similar
Multimode or luminescence plate reader	Refer to the Instrument Compatibility Chart at discoverx.com/instrument-compatibility/
Sterile disposable reagent reservoir	Thermo Fisher Scientific, Cat. No. 8094, or similar
50 mL and 15 mL Polypropylene tubes, sterile	
1.5 mL polypropylene microcentrifuge tubes, sterile	
Tissue culture disposable pipettes (1 mL - 25 mL), sterile	
Disposable pipette tips for P20, P100, P1000 pipettes	
Sterile biosafety cabinet	
Automated cell counter or hemocytometer	
Humidified tissue culture incubator	
Single and multichannel pipettes (e.g. P20, P100, P1000)	

Note: *A positive Control is only required for kits purchased with the bioassay kit configuration that does not include it. The Positive Control is used for QC testing of the bioassay cells in this kit, as reflected in the Certificate of Analysis.

6. Unpacking Cell Cryovials

Cryovials are shipped on dry ice and contain cells in 0.2mL of AssayComplete™ Freezing Reagent. Upon receipt, the vials should be transferred to the vapor phase of liquid nitrogen for storage beyond 24 hours. The following procedures are for the safe storage and removal of cryovials from liquid nitrogen storage.

Appropriate personal protective equipment (PPE), including a face shield, gloves, and a lab coat should be worn during these procedures.

1. cAMP Hunter CHO-K1 GLP1R Bioassay Cells must arrive in a frozen state on dry ice.

Contact technical support immediately, if cells received were already thawed.

2. Frozen cells must be transferred to liquid nitrogen storage or a -80°C freezer immediately upon arrival. If cells will be thawed and used within 24 hours, they can be stored temporarily at -80°C. Place vials in the vapor phase of liquid nitrogen storage for long-term storage.

Cryovials should be stored in the vapor phase of liquid nitrogen ONLY. These vials are not rated for storage in the liquid phase.

3. When ready to perform the assay, using tongs, remove the cryovials from the liquid nitrogen storage, and place them immediately in dry ice, in a covered container. Wait for at least one minute for any liquid nitrogen inside the vial to evaporate.

4. Proceed with the thawing protocol in the detailed assay protocol section 8.1.

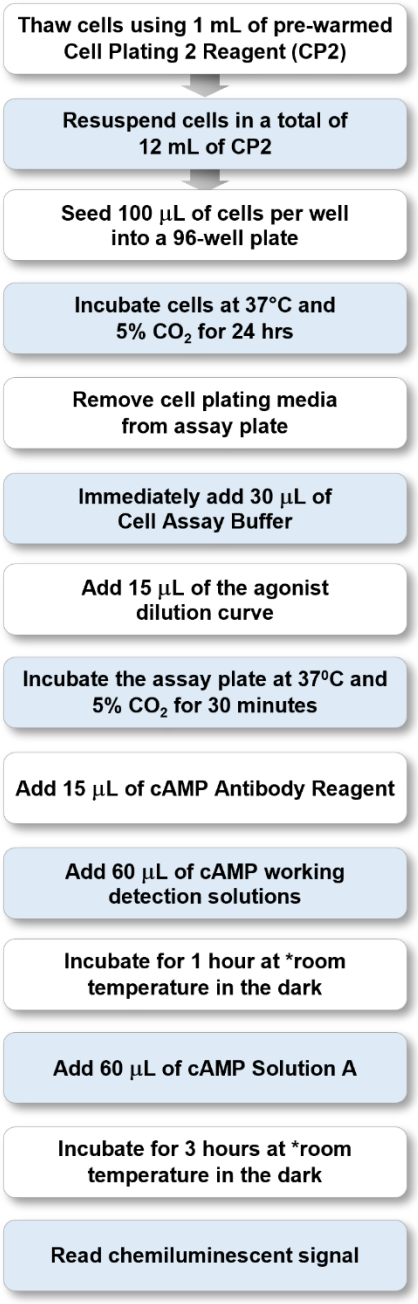
7. Protocol Schematic

Quick-start Procedure: In a 96-well plate, perform the following steps.

Tip: *Use this sheet to note your assay specific conditions. Post it on your bench to use as a quick reference guide.*

Assay Name: _____Date: _____

Product Details: _____



*Room temperature refers to a range of 23-25°C

8. Detailed Protocol

This assay is performed under aseptic conditions. Cells and reference standards/test samples are prepared in a Biological Safety Cabinet following good aseptic technique. All appropriate materials are either certified sterile or prepared aseptically.

Prepared volumes may be scaled up or down if required. If purchasing the bioassay kit without control, it can be sourced per the details in the Additional Materials and Equipment Recommended for Assay.

8.1 Bioassay Cell Preparation

(Day 1)

The following protocol is for thawing and plating cryopreserved CHO-K1 GLP1R bioassay cells from cryovials.

1. Before thawing the cells, ensure all required materials are set up in the biosafety cabinet. These include:
   a. One sterile 25 mL reagent reservoir
   b. One sterile 15 mL conical tube
   c. A P1000 pipette set to dispense 1 mL
   d. A multichannel pipette and tips set to dispense 100 µL
   e. An aliquot of AssayComplete™ Cell Plating 2 Reagent (CP2), **pre-warmed in a 37°C water bath for 15 minutes and equilibrated to room temperature.**
   f. A 96-Well White, Clear bottom, Tissue Culture-Treated Sterile Assay Plate (provided with the kit)
2. Dispense 12 mL of CP2 into the 15 mL conical tube.
3. Remove the cryovial from the liquid nitrogen storage and immediately place it in dry ice to return to lab.
4. Remove the cryovial from dry ice and ensure the cap is tightened. Immediately thaw the vial in a 37°C water bath for 30 (+/- 5) seconds.
   DO NOT LEAVE IN WATER BATH!
5. Visually inspect the bottom of the vial after 20 seconds. If the pellet is thawed, remove the vial from the water bath, wipe down the outside surface quickly with 70% ethanol, and immediately bring it into the tissue culture hood. If ice is still visible, return the vial to the water bath for an additional 10-15 seconds.
6. Add 1.0 mL of pre-warmed CP2 from the 15 mL conical tube into the cryovial. The reagent should be added slowly along the wall of the cryovial tube. Slowly pipette the cell suspension up and down, several times, to uniformly resuspend the cells.
7. Transfer the cell suspension to the conical tube containing the remaining 11 mL of CP2. Remove any remaining liquid from the cryovial to ensure maximum cell recovery.
8. Replace the cap on the conical tube and mix by gentle inversion 2-3 times to ensure that the cells are resuspended adequately in the reagent without creating any froth in the suspension. Immediately

transfer the suspension into the sterile 25 mL reagent reservoir.

9. Using a manual 12-channel multichannel pipette, transfer 100 µL of the cell suspension to each well of the 96-well assay plate one row at a time, using reverse pipetting. Mix cells in the reagent reservoir by pipetting up and down 2-3 times before aspirating and dispensing cells into each subsequent row in the assay plate.
10. Replace the lid and leave the plate at room temperature for 15 minutes to allow the cells to settle uniformly in the well and minimize potential edge effects.
11. Incubate the assay plate in a humidified tissue culture incubator at 37°C and 5% CO_2 for 24 ± 2 hours before proceeding with the assay.

8.2 Sample Preparation

(Day 2)

Exendin-4 Control Agonist Preparation

The following protocol gives the recommended dilutions for preparing the Exendin-4 control agonist working stocks and serial dilutions.

1. Before beginning, ensure all required reagents are assembled in the biosafety cabinet:
    a. Pipettes (P20, P100, P1000)
    b. A multichannel pipette and tips set to dispense 20 µL.
    c. An aliquot of AssayComplete™ Protein Dilution Buffer-B2, **pre-warmed in a 37˚C water bath for 15 minutes, then equilibrated to room temperature**.
    d. A 96-Well Green, V-Bottom, Untreated, Non-Sterile Dilution Plate, labeled ‘Master dilution plate’ (MDP)
    e. Sterile microfuge tubes, for preparing intermediate, working, or nominal testing concentrations of reference or test samples.
2. On the day of assay, prepare Exendin-4 intermediate and working stock solutions in PDB-B2 as shown in examples in Table 1 below.
    a. Exendin-4 control (see Additional Materials Required table if the kit was purchased without control) is supplied as a lyophilized powder and is dissolved in 0.478 mL of supplied reconstitution buffer (PDB) (as recommended in datasheet) to prepare a 1.046 mg/mL stock concentration.
    b. Reconstituted Exendin-4 stock from 2a may be stored in small aliquots at -20°C. Avoid multiple freeze-thaws.
    c. It is recommended to use a minimum aliquot size of 10 µL for the preparation of all sample/stock dilutions.

Table 1. Example of Preparation of Exendin-4 Intermediate Dilutions & Working Stock

Intermediate and Working Stock Solutions	Final Concentration, ng/mL	Volume of Exendin-4 (Stock concentration)	Volume Dilution Buffer (PDB-B2), µL
Exendin-4, Intermediate Stock 1	20,840	10 µL of 1.046 mg/mL	492
Exendin-4, Intermediate Stock 2	1,040	15 µL of 20,840 ng/mL	285
Exendin-4, Intermediate Stock 3	52	15 µL of 1,040 ng/mL	285
Working Stock of Exendin-4	3	23 µL of 52 ng/mL	377

Note: Use a vortex to mix (10-20 sec) for each dilution before preparing subsequent serial dilution.

3. On the day of the assay, prepare serial dilutions of the Exendin-4 control in the master dilution plate (MDP) at 3X the final concentrations of each dilution in PDB-B2 (i.e., the Ligand Diluent).

   Table 2 below indicates an example sample dilution scheme. Following this scheme should result in sufficient volumes to run triplicate wells per dose in the assay plate.

   a. Add the appropriate volume of PDB-B2 to Row A, Well 1 of the MDP, as indicated in column 6 of Table 2.

   b. Transfer the appropriate volume (indicated in column 5, Table 2) of Exendin-4 Working Stock (3X stock = 3 ng/mL or 3,000 pg/mL) to the MDP Row A, Well 2.

   c. Transfer the indicated volume (column 5, Table 2) of Exendin-4 from the source well (indicated in column 4) to the destination well (indicated in column 1). Pipette up and down several times after each transfer to ensure dilutions are mixed well. Replace pipette tips between each serial dilution.

   d. No Exendin-4 is added to Row A Well 1 (PDB-B2 only) to serve as the negative control.

Table 2. Sample concentration in Master Dilution prepared as a 3X stock: Example of Preparation of Exendin-4 control Serial Dilutions

Column 1	Column 2	Column 3	Column 4	Column 5	Column 6	Column 7
Well location on Master Dilution Plate (MDP)	Concentration (3X) of Exendin-4 on MDP Row A, pg/mL	Dilution Factor	Dilution (3X) Exendin-4 Source Well	Volume (3X) of sample added, µL	Volume of Ligand Diluent, µL	Final Concentration (1X) of sample in Assay Plate, pg/mL
Row A, Well 1	0	--	--	--	180	--
Row A, Well 2	3000	--	Working Stock (3 ng/mL) from Table 3	300	-	1000
Row A, Well 3	1000	3	Row A, Well 2	100	200	333
Row A, Well 4	333	3	Row A, Well 3	100	200	111
Row A, Well 5	111	3	Row A, Well 4	100	200	37
Row A, Well 6	74	1.5	Row A, Well 5	200	100	24.7
Row A, Well 7	49.3	1.5	Row A, Well 6	200	100	16.5
Row A, Well 8	32.9	1.5	Row A, Well 7	200	100	11
Row A, Well 9	21.9	1.5	Row A, Well 8	200	100	7.3
Row A, Well 10	11	2	Row A, Well 9	150	150	3.7
Row A, Well 11	3.7	3	Row A, Well 10	100	200	1.2
Row A, Well 12	1.2	3	Row A, Well 11	100	200	0.4

4. On the day of assay, prepare appropriate dilutions of Test Sample (TS) stocks (e.g., intermediate dilutions), as needed, to generate an appropriate working stock concentration from which to prepare serial dilutions.
   a. Stock solutions and serial dilutions should be prepared in PDB-B2 diluent.
   b. For preparing all sample/stock dilutions, it is recommended that a minimum aliquot size of 10 µL of concentrated stock solutions be used.

Note: optimal dilution range of test sample should be empirically determined

   c. Prepare serial dilutions of the test sample in row B of the master dilution plate.

5. Assay plate preparation:
   a. Remove assay plates with cells from the incubator.
   b. With a flicking motion, remove liquid from the assay wells in the plate. Then, place the assay plate upside down on a lab-grade low lint tissue/paper towel and spin in a plate centrifuge set at 1000 rpm. Stop once it reaches 1000 rpm. Alternatively, completely remove the Cell Plating Reagent from each well in the assay plate by carefully aspirating the medium.

c. Immediately add 30 µL of Cell Assay Buffer to all wells of the plate.

6. Transfer 15 µL of the 3X sample dilutions from the MDP to the appropriate wells containing cells in the assay plate.
   Refer to Figure 3. Representative Assay Plate Map:
   a. Row A in MDP: transfer to Rows B, D, and F in the assay plate (Exendin-4 control)
   b. Row B in MDP: transfer to Rows C, E, and G in the assay plate (Test Sample)

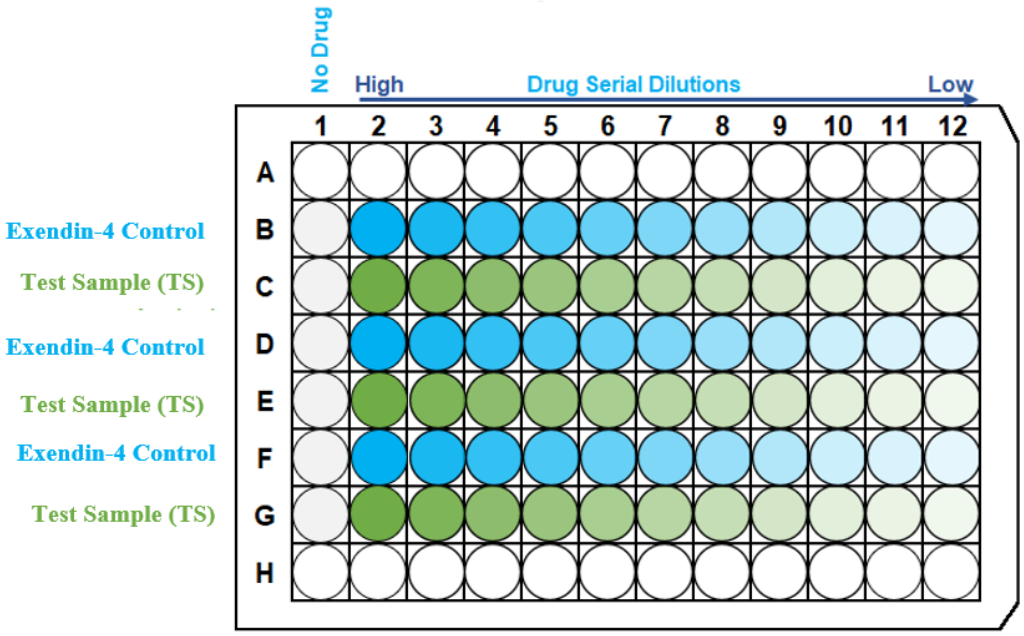


Figure 2. Representative Assay Plate Map

This plate map shows two interdigitated 11-point dose curves, with three replicates per dose point, for a test sample (TS) and reference standard (RS) prepared using the same dilution scheme. Column 2 contains the highest dose of each sample, while column 12 contains the lowest dose. Column 1 contains no drug (PDB-B2 only).

7. Incubate the assay plate in a humidified incubator at 37°C and 5% CO_2 for 30 minutes.

8.3 Addition of Detection Reagent

Signal Detection

1. During the 30-minute agonist incubation period, prepare the cAMP working detection solution in a separate 15 mL polypropylene tube by mixing 19 parts of cAMP Lysis Buffer, 5 parts of Substrate Reagent 1, 1 part Substrate reagent 2, and 25 parts of cAMP Solution D.

Refer to Table 3 below for the volume of each component required for one 96-well plate. Adjust volumes according to your assay set-up, keeping the ratios consistent. Store in the dark before use.

Table 3. Working Detection Solution Preparation

Working Detection Solution		
Components	**Volume Ratio**	**Volume per 96-well Plate (mL)**
cAMP Lysis Buffer	19	3.8
Substrate Reagent 1	5	1.0
Substrate Reagent 2	1	0.2
cAMP Solution D	25	5.0
Total Volume		**10**

2. Following agonist incubation, add 15 µL of the cAMP Antibody Reagent to all wells of the assay plate.
3. Add 60 µL of the prepared cAMP working detection solution to all wells of the assay plate. **Do not pipette up and down or vortex the plate to mix**.
4. Incubate the assay plate for 1 hour at room temperature in the dark.

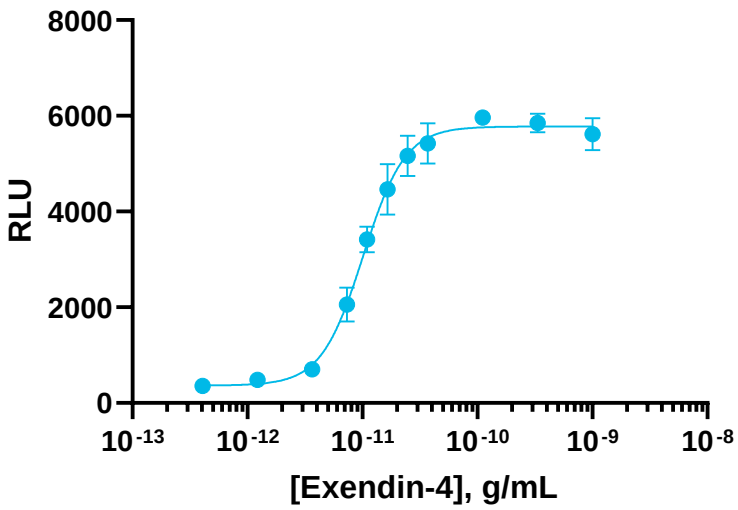
Room temperature refers to a range of 23-25°C.

5. Add 60 µL of cAMP Solution A to all wells of the assay plate. **Do not pipette up and down or vortex the plate to mix**.
6. Incubate the assay plate for 3 hours at room temperature in the dark.
7. Read the sample on a Perkin Elmer Envision, with a 0.2 sec/well integration time, or on a similar benchtop instrument. Refer to the Instrument Compatibility Chart at discoverx.com/instrument-compatibility/
8. Data analysis can be performed using any statistical analysis software, such as GraphPad Prism, SoftMax Pro, Gen5, Microsoft Excel, or other preferred software.

9. Typical Result

The following graph is an example of a typical dose-response curve for the GLP-1 RA Bioassay generated using the protocol outlined in this manual. The data shows a potent, dose-dependent increase in cAMP production when treated with Exendin-4. The plate was read on the EnVision® Multimode Plate Reader, with a 0.2 sec/well integration time, and data analysis was conducted using GraphPad Prism.

A



B

S/B	EC_{50}, pg/mL
17	10.4

Figure 3. Representative A, dose-response curve, and B, EC_{50} and assay window (Signal/Background, S/B) for Exendin-4-mediated GLP-1R activation, as measured in this bioassay.

10. Troubleshooting Guide

Problem	Potential Cause	Proposed Solution
No response	Incorrect thawing procedure	Refer to the thawing instructions in the Bioassay Cell Preparation section of this user manual.
	Incorrect ligand used or improper ligand incubation time	Refer to the Certificate of Analysis for recommended ligand and assay conditions.
	Incorrect preparation of the ligand (agonist or antagonist)	Refer to the vendor-specific datasheet to ensure proper handling, dilution, and storage of the ligand.
	Sub-optimal time course for induction	Optimize the time course of induction with agonist and antagonist.
Decreased or no response	Cells are not adherent and exhibit incorrect morphology	Confirm adherence of cells using a microscope.
	Problem with the microplate reader	The microplate reader should be in luminescence mode. Read at 0.1-1 seconds/well.
Low or no signal	Incorrect preparation of detection reagents	Detection reagents are light-sensitive and should ideally be prepared just prior to use.
	Problem with a microplate reader	The microplate reader should be in luminescence mode. Read at 0.1-1 second/well.
Experimental S/B does not match the value noted in the Certificate of Analysis provided	Incorrect incubation temperature	Confirm assay conditions. Check and repeat the assay at the correct incubation temperature, as indicated on the assay datasheet.
	Incorrect preparation of ligand (agonist or antagonist)	Some ligands are difficult to handle. Confirm the final concentration of ligands.
EC_{50} is right-shifted	Improper ligand handling or storage Difference in agonist binding affinity	Ensure that the ligands are stored and incubated at the proper temperature. Confirm that the ligand used is comparable to the ligand in the Certificate of Analysis.
	Problems with dynamic range or dilutions	Changing tips during dilution can help in avoiding carryover. Include a cAMP standard curve in the assay to ensure that the ligand dose curve is in the dynamic range of the cAMP detection kit. Moving out of the dynamic range may shift the EC_{50} of the ligands.
High variability between replicates	Instrument calibration	Ensure that the dispensing equipment is correctly calibrated and proper pipetting technique is used.

For questions on using this product, please contact Technical Support at discoverx.com/support/

Document Revision History

Revision Number	Date Released	Revision Details
0	May 2024	New Document

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