# GLP1 NAb Assay: A Case Study for Simple, Serum-tolerant Cell-based Assays to Detect Neutralizing Antibodies to Biosimilar and Bioinnovator drugs

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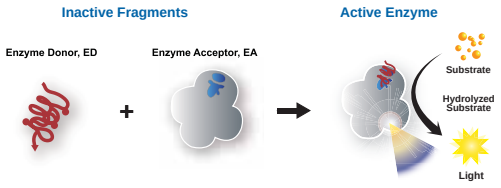
## Abstract

Neutralizing antibodies (NAbs) to biological drugs may cause loss of therapeutic efficacy and in some cases, loss of endogenous protein function as well. Standard immunoassays can detect anti-drug antibodies, but cannot differentiate neutralizing antibodies, so cell-based assays are often required by the regulatory agencies to identify NAbs. Therefore, a key step in the development of a biotherapeutic is the selection and development of an appropriate cell-based bioassay for NAbs, which has traditionally posed significant challenges, especially regarding serum/matrix tolerance, sensitivity and specificity.

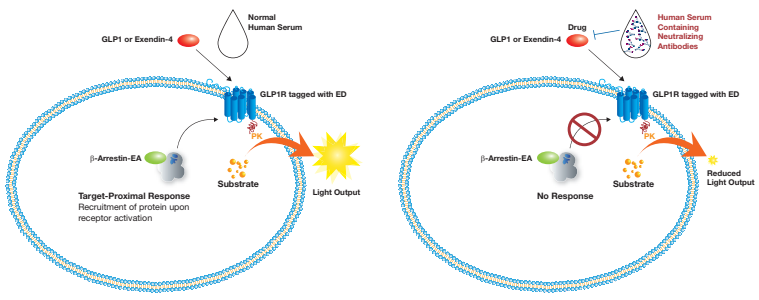
Here, we describe the development, validation and application of PathHunter® cell-based assays, a novel technology platform to create simple cell-based assays for the detection of NAbs to biological drugs. This technology relies on cells expressing full length, native receptors to create assays that are highly specific, robust and have a homogenous mix-and-read protocol. This enables accurate and sensitive detection of neutralizing antibodies even in high concentrations (up to 100%) of human serum through a simple chemiluminescent output. The assays are developed in thaw-and-use format to increase convenience and minimize assay variability due to cell culture and handling. The cell preparation, bioassay protocol and reagents have been optimized to provide superior bioassay performance with high reproducibility (<7% RSD). A case study will be presented on the GLP1 receptor assay, as an example of the multitude of assays available for bioinnovator and biosimilar drugs.

## Enzyme Fragment Complementation



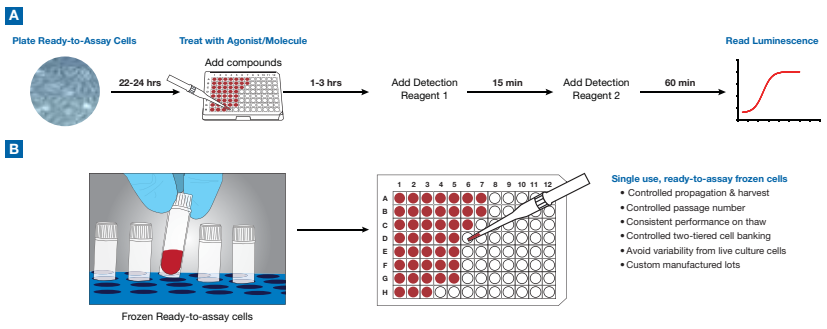

DiscoveRx's proprietary PathHunter Enzyme Fragment Complementation (EFC) technology consists of the β-galactosidase (β-gal) enzyme, split into two inactive components, the enzyme donor peptide (ED) and enzyme acceptor (EA). When brought together in close proximity, ED complements with EA forming active β-gal. The active enzyme catalyzes the substrate generating chemiluminescent light, providing a highly amplified signal and thus an assay of high sensitivity.

## GLP1R NAb Assay With β-Arrestin Readout



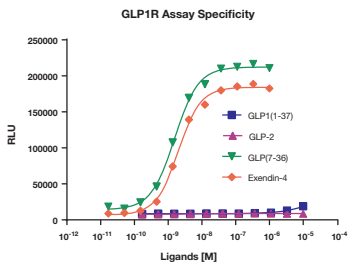

Tapping into the natural biology of the GLP1R receptor, PathHunter EFC technology has been used to create simple, cell-based chemiluminescent assays for receptor activation. The activation of the ED-tagged GLP1 receptor results in the recruitment of EA-tagged β-Arrestin. This forces the complementation of the EFC components, EA and ED, resulting in the formation of a functional enzyme that hydrolyzes available substrate and generates a chemiluminescent signal. The signal generation is unaffected by the presence of human serum or plasma. Only when neutralizing antibodies are present in the serum or plasma matrix that specifically inhibit GLP1 or Exendin-4 function, the GLP1 receptor is not activated, and there is reduced chemiluminescent signal from the assay.

## A Simple Homogenous Protocol With Rapid Results




PathHunter bioassay kits use a simple homogenous protocol with rapid results. (**A**) Ready-to-assay cells from DiscoveRx are plated on a 96-well plate or 384-well plate and incubated for about 24 hours at 37°C. The agonist/test molecule is added to the plate and incubated for 1-3 hours. The detection reagents are added sequentially in two addition steps and the chemiluminescent signal can be detected on any plate-reading luminometer. (**B**) Cells manufactured in bioassay kits are meant for single use in ready-to-assay vials. The frozen cells are plated directly onto plates to run the assay and this format has several advantages as outlined above.

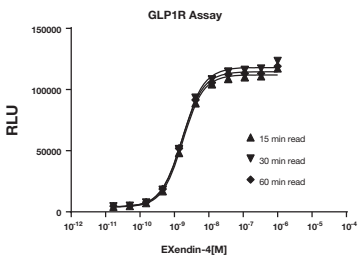
## PathHunter Bioassays are Highly Specific




| | GLP1(1-37) | GLP-2 | GLP(7-36) | Exendin-4 |
|---|---|---|---|---|
| HillSlope | 0.8982 | 0.9092 | 1.237 | 1.317 |
| $EC_{50}$ | 4.887e-005 | 2.869e-009 | 1.564e-009 | 2.011e-009 |
| S/B | | | 15.0 | 26.0 |

Assay signal is dependent on the presence of appropriate agonist. The assay detects GLP(7-36) and exendin-4, both of which are known agonists for the GLP1R. No assay signal is observed for GLP1(1-37) and GLP2, known not to activate the receptor.

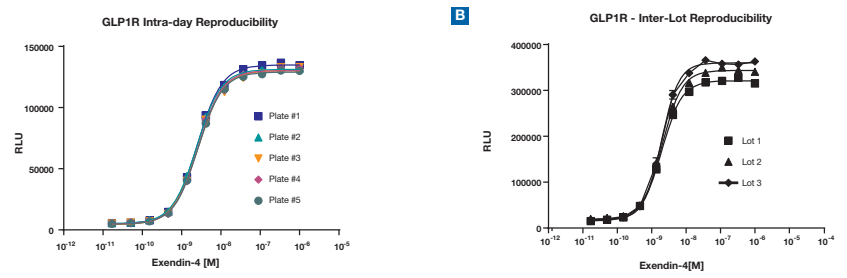
## Robust Bioassays with Flexible Protocols




| Run | Incubation Time (min) | $EC_{50}$ | S:B Ratio |
|---|---|---|---|
| 1 | 15 | 1.756 nM | 28 |
| 2 | 30 | 1.7049 nM | 29.1 |
| 3 | 60 | 1.671 nM | 27.5 |

PathHunter bioassay protocols are robust. The incubation time was varied after detection reagent 2 was added to the well. As observed, there was no noticeable change in the output of the assay from 15 to 60 minutes of incubation, indicating that the assay is resilient to slight modifications in the protocol.

## GLP1R Bioassays are Highly Reproducible




| | Plate #1 | Plate #2 | Plate #3 | Plate #4 | Plate #5 |
|---|---|---|---|---|---|
| HillSlope | 1.393 | 1.395 | 1.365 | 1.423 | 1.396 |
| $EC_{50}$ | 2.533e-009 | 2.426e-009 | 2.610e-009 | 2.616e-009 | 2.609e-009 |
| S/B | 27.2 | 25.3 | 29.7 | 26.4 | 27.5 |

| | Lot 1 | Lot 2 | Lot 3 |
|---|---|---|---|
| HillSlope | 1.465 | 1.506 | 1.699 |
| $EC_{50}$ | 1.958e-009 | 2.029e-009 | 1.920e-009 |
| S/B | 20.4 | 17.6 | 19.5 |

**C**

| GLP1R Inter-day and Inter-operator Reproducibility | | | | | | |
|---|---|---|---|---|---|---|
| | | Plate #1 | Plate #2 | Plate #3 | Plate #4 | Plate #5 |
| Day 1 Operator 1 | Avg %CV | 4.23 | 4.07 | 3.97 | 3.88 | 3.95 |
| | S:B | 29.96 | 29.44 | 31.2 | 31.8 | 29.9 |
| | $EC_{50}$ | 2.43E-09 | 2.30E-09 | 2.27E-09 | 2.43E-09 | 2.47E-09 |
| Day 2 Operator 2 | Avg %CV | 3.22 | 3.95 | 3.79 | 3.77 | 4.82 |
| | S:B | 25.48 | 24.71 | 24.7 | 24.8 | 24.88 |
| | $EC_{50}$ | 2.36E-09 | 2.28E-09 | 2.20E-09 | 2.21E-09 | 2.19E-09 |

PathHunter bioassays are highly reproducible. (**A**) Intra-day and intra-lot variability was tested with 5 different plates and 5 different vials of frozen ready-to-assay cells. (**B**) Inter-lot variability was tested with 3 different lots of cells produced at different times, tested on the same plate. (**C**) Day to day and operator variability was tested by looking at two different operators on two separate days. As we observe, the assay demonstrated a high reproducibility with 3-7 % CV.

## GLP1R NAb Assay With cAMP Readout

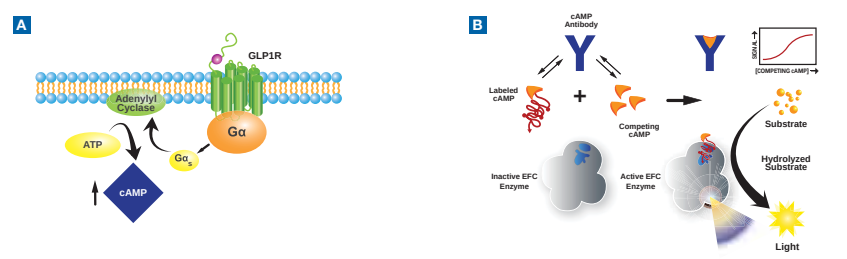
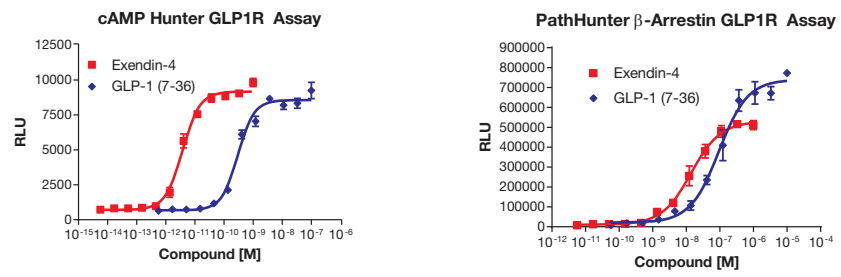



Figure A. Fully optimized functional assay for GLP1R to detect cAMP second messenger signaling, including the cells and cAMP detection reagents. Figure B. HitHunter cAMP Assay Principle. Free cAMP from cell lysates competes for antibody binding against labeled cAMP (ED-cAMP conjugate). Unbound ED-cAMP is free to complement EA to form active enzyme by EFC, which subsequently hydrolyzes substrate to produce signal. A positive signal generated is directly proportional to the amount of free cAMP bound by the cAMP antibody.

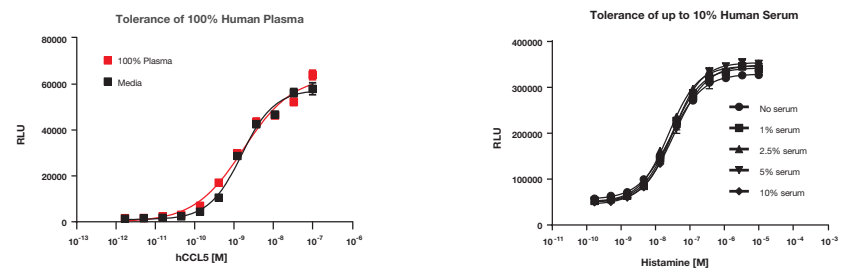
## GLP1R cAMP & β-Arrestin Assays Respond to Exendin-4 and GLP1 (7-36)




| | Exendin-4 | GLP-1 (7-36) |
|---|---|---|
| $EC_{50}$ | 3.770e-012 | 2.986e010 |
| S/B | 12.5 | 13.0 |

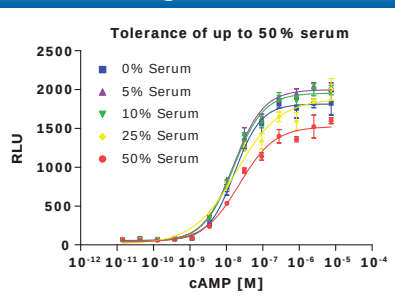
| | Exendin-4 | GLP-1 (7-36) |
|---|---|---|
| $EC_{50}$ | 1.392e-008 | 9.420e-008 |
| S/B | 41.7 | 38.9 |

## PathHunter NAb Assays Are Tolerant of Up To 100% Human Serum/Plasma




PathHunter assays have a high tolerance of human serum. PathHunter NAb assays can detect functional biologics in high concentrations of human serum and human plasma. This data and other published work (Ryding *et al.*, 2013) demonstrate that PathHunter assays give an unwavering response in up to 100% human serum and human plasma. This has enabled the use of these for both direct and indirect NAb assays.
Ryding *et al*., 2013. *J Immunological Methods*.

## HitHunter cAMP Assays Are Tolerant Of High Concentrations Of Serum




HitHunter cAMP Assays Are Tolerant Of High Concentrations Of Serum. The cAMP standard was diluted in increasing amounts of serum. The data indicates that high amounts of serum can be tolerated by the HitHunter cAMP assay indicating its suitability for use in NAb assay development.

## Summary & Conclusions

- Assays for GLP1 variants and Exendin-4 are available in both cAMP and β-Arrestin readouts
- PathHunter GLP1R assays provide a target-specific response and can tolerate up to 100% human serum or plasma
- GLP1R assays are robust and highly reproducible with a simple, homogenous protocol
- GLP1R assays presented have been used extensively for potency assays and NAb assays
- To minimize assay variability, the GLP1R bioassays are prepared in ready-to-assay kits with all required assay components, including frozen, single use cell vials
  - Cells in ready-to-assay format minimize assay variability stemming from the use of continuous culture cells
  - Each kit contains enough material for 1000 datapoints (10 x 96-well plates)
  - Customized lots of up to 1000 vials are prepared for each project
- Similar assays are available for >700 targets, including >30 Biosimilar targets

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