Validated Bioassays for Potency, Stability and Lot Release Testing of Biopharmaceuticals

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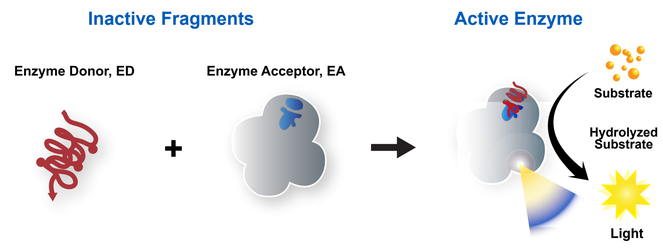
Abstract

Biologics represents one of the fastest growing classes of biopharmaceutical molecules today. One of the major bottlenecks in the development of biologics is the lack of ready-to-use bioassays for potency, stability and lot release testing. Ideal bioassays need to reflect the clinical mechanism of action (MOA) of the biopharmaceutical drug and should be simple, accurate, precise, reproducible and robust according to proposed ICH guidelines.

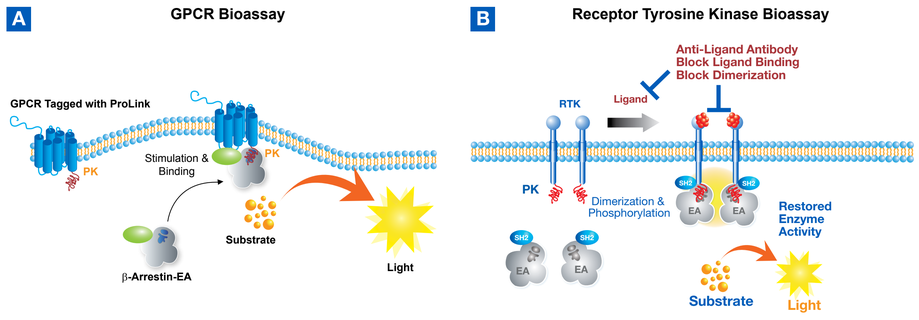
We describe the validation and application of PathHunter® cell-based assays, a novel technology platform to create simple bioassays with rapid and reproducible results. This technology utilizes cells expressing full length receptors to pinpoint the underlying native biology of the receptor and drug, thereby reflecting the clinical MOA of the biopharmaceutical drug. These assays are precise, scalable, robust and have a homogenous mix-and-read protocol, which facilitates accurate detection of drug potency. These target-specific assays have been developed for a majority of GPCRs, RTKs, Cytokine receptors and a variety of other receptors. Assays for GLP1R are presented as case studies, demonstrating the validation and application of these assays for potency, stability and lot-release testing of biopharmaceutical drugs.

PathHunter® Enzyme Fragment Complementation



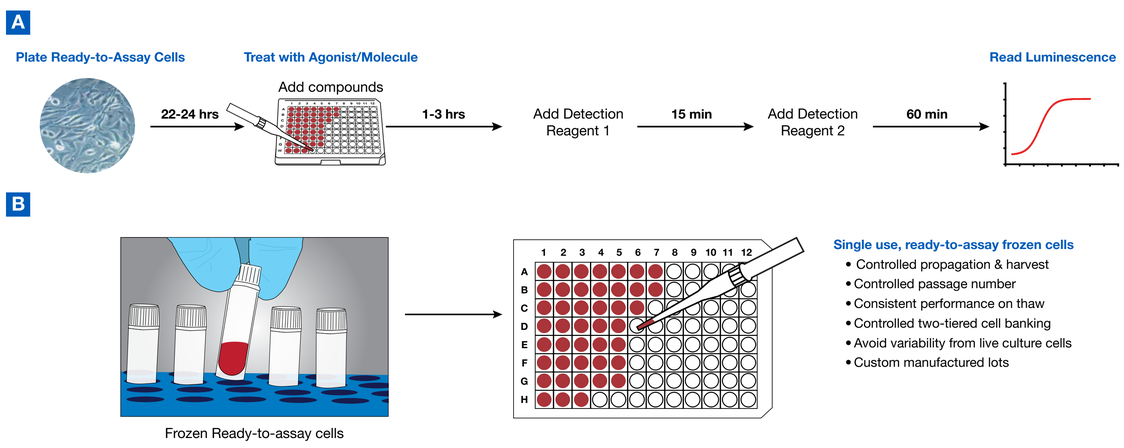
DiscoveRx's proprietary PathHunter Enzyme Fragment Complementation (EFC) technology consists of the β-galactosidase (β-gal) enzyme, split into two inactive components, the enzyme donor peptide (ED) and enzyme acceptor (EA). When brought together in close proximity, ED complements with EA forming active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

Introduction to PathHunter Bioassays



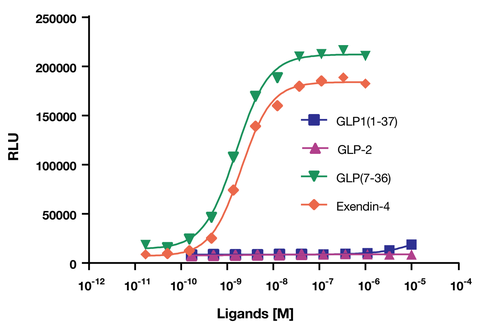
Tapping into the natural biology of receptors, PathHunter EFC technology has been used to create simple, cell-based chemiluminescent assays. (**A**) **PathHunter GPCR cell-based assay**. Activation of the ED-tagged GPCR results in β-Arrestin recruitment and formation of a functional enzyme capable of hydrolyzing substrate and generating a chemiluminescent signal. (**B**) **PathHunter RTK cell-based assay**. Activation of the ED-tagged RTK results in the recruitment of an SH2-domain containing protein and formation of a functional enzyme capable of hydrolyzing substrate and generating a chemiluminescent signal.

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.

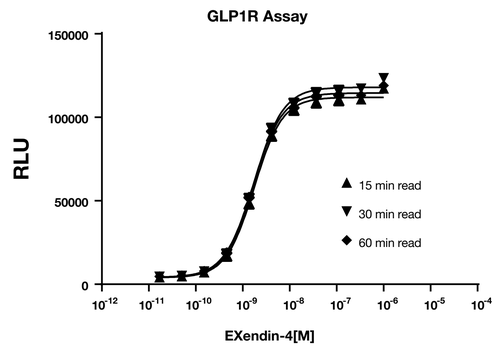
PathHunter Bioassays are Highly Specific



	GLP(7-36)	Exendin-4
HillSlope	1.237	1.317
EC_{50}	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, is not 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.

PathHunter Bioassays are Highly Reproducible

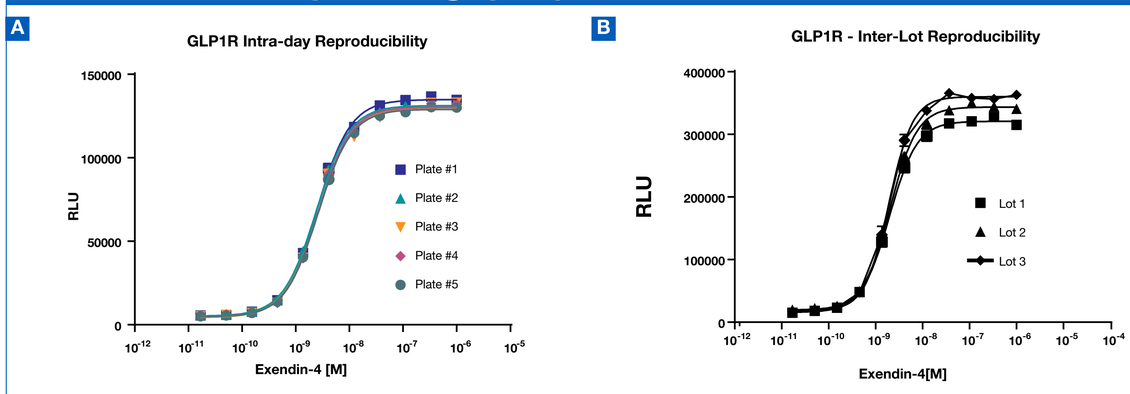


	Plate #1	Plate #2	Plate #3	Plate #4	Plate #5
HillSlope	1.393	1.395	1.365	1.423	1.396
EC_{50}	2.533e-009	2.426e-009	2.610e-009	2.616e-009	2.609e-009
S/B	27.2	25.3	29.7	26.4	27.5

	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

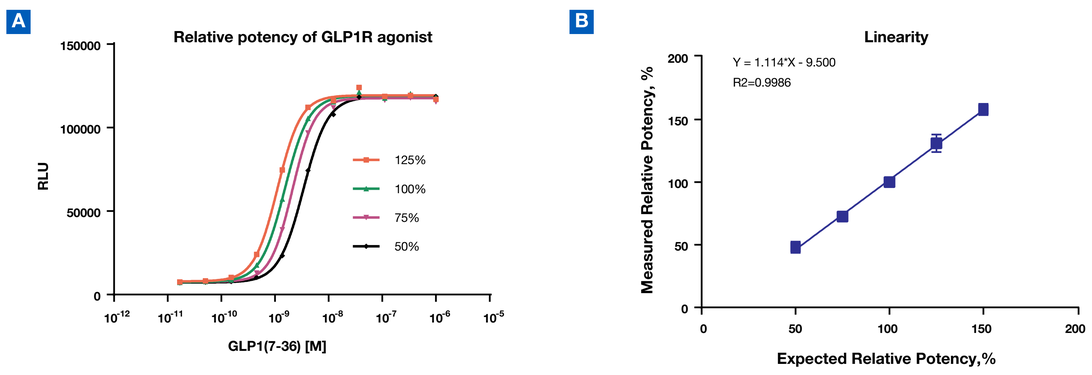
C

GLP1R Intra-day 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 observed, the assay demonstrated a high reproducibility with 3-7 % CV.

Measurement of Relative Potency with PathHunter Bioassays



Run	Expected Relative Potency	EC_{50}	Measured Relative Potency
1	50%	3.371 nM	45%
2	75%	2.110 nM	72%
3	100%	1.528 nM	100%
4	125%	0.940 nM	136%

PathHunter bioassays have been used to measure relative potency. Parallelism and relative potency of a reference standard can be measured with PathHunter assays. The GLP1R assay was tested with reference standards from 50% to 150%. The dose response curves can be seen in (**A**). The measured relative potencies were plotted against the expected relative potencies (**B**) with a very high degree of accuracy.

Summary & Conclusions

- PathHunter cell-based bioassays are chemiluminescent assays, reflective of receptor native biology
- Can be used to quantify potency & stability of biotherapeutics
- Biotherapeutics tested include novel receptor agonists, anti-ligand antibodies and anti-receptor antibodies
- PathHunter assays are specific, robust, reproducible, linear and precise
- A simple and homogenous protocol with rapid results
- Bioassay kits with frozen ready-to-assay cells provide low variability between assay runs
- Suitable for lot release, stability studies and characterization of biotherapeutics

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