Development of Novel Cell-based Assays to Detect Neutralizing Antibodies to Therapeutic Biologics

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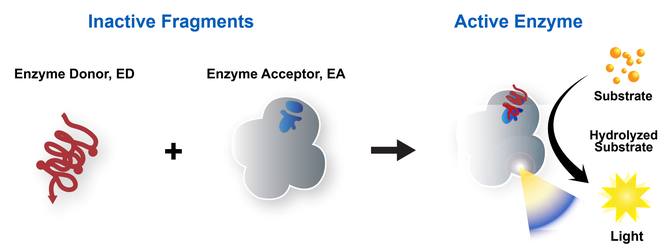
Abstract

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 necessary 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 tolerance, sensitivity and specificity.

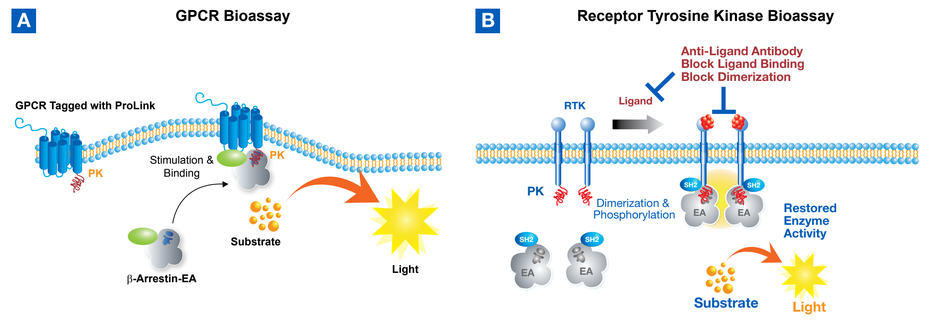
We describe the development, validation and application of PathHunter® cell-based assays, a novel technology platform to create simple cell-based assays for the detection of NAbs to biological drugs. This technology relies on cells expressing full length, native receptors to create assays that are highly 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 technology behind the assay and of case studies will be presented, demonstrating the utility of these cell-based assays for detecting NAbs for a wide variety of biological drugs ranging from proteins, peptides to MAbs and bi-specific antibodies to a variety of targets.

PathHunter® Enzyme Fragment Complementation



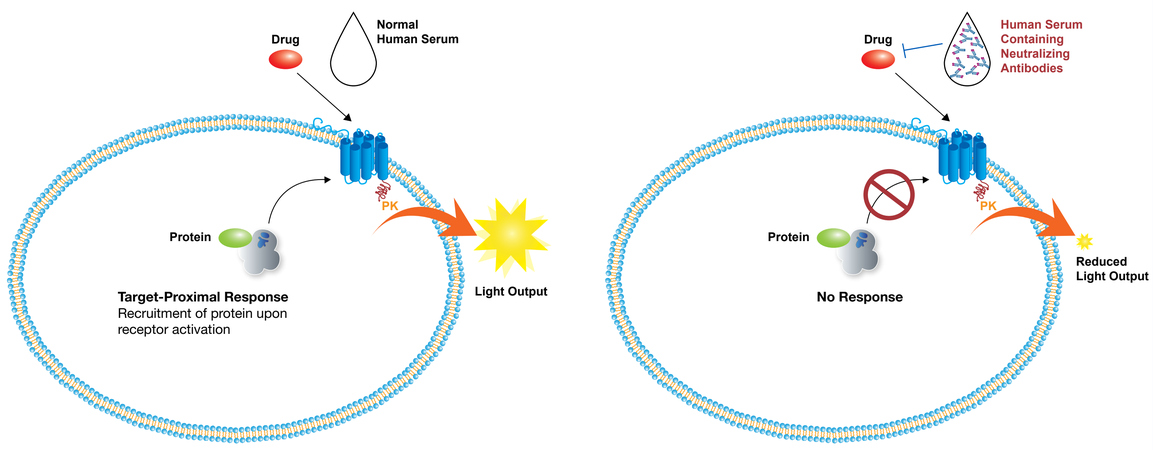
DiscoveRx's proprietary PathHunter Enzyme Fragment Complementation (EFC) technology consists of the β-galactosidase (β-gal) enzyme, split into two inactive components, the enzyme donor peptide (ED) and enzyme acceptor (EA). When brought together in close proximity, ED complements with EA forming active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

PathHunter Assays Are Reliant on Native Receptor Biology

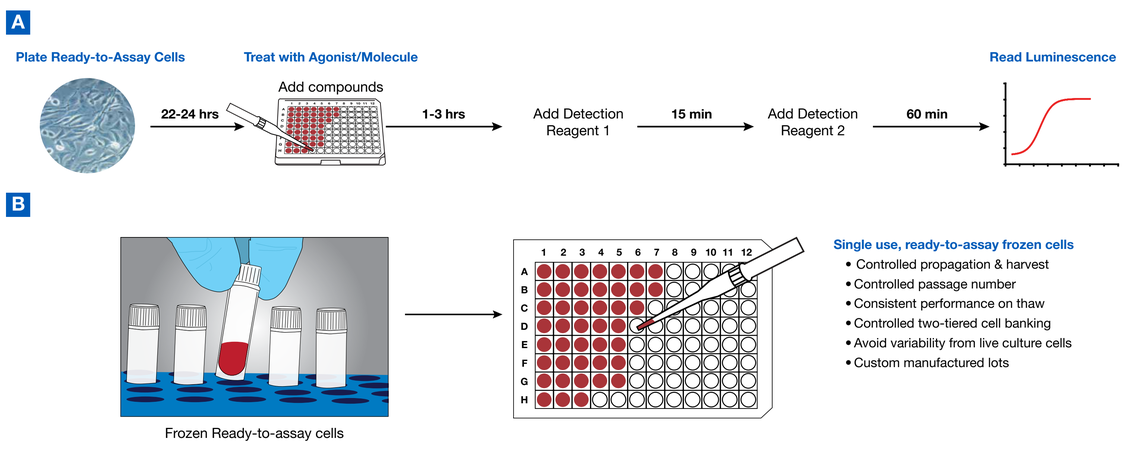


Tapping into the natural biology of receptors, PathHunter EFC technology has been used to create simple, cell-based chemiluminescent assays. (**A**) **PathHunter GPCR cell-based assay**. Activation of the ED-tagged GPCR results in β-Arrestin recruitment and formation of a functional enzyme capable of 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 Chemiluminescent Assay to Detect Neutralizing ADAs

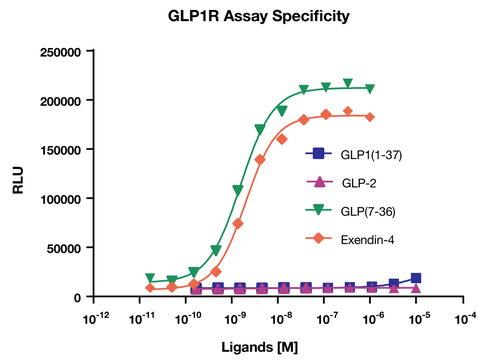


A Simple Homogenous Protocol With Rapid Results



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.

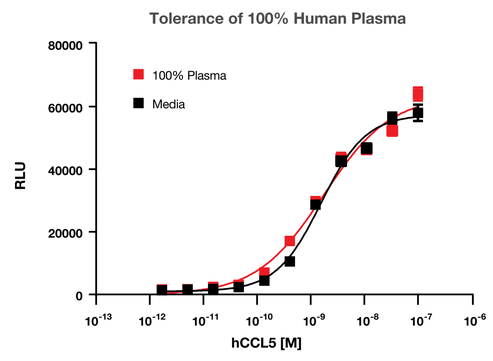
PathHunter Bioassays are Highly Specific

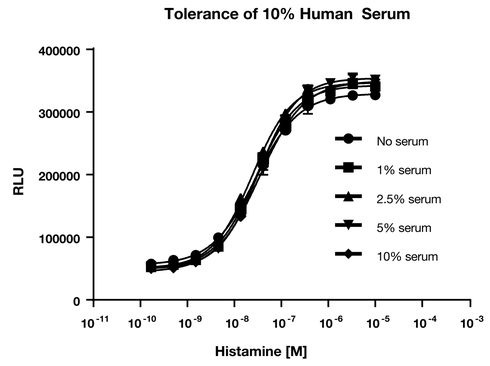


	GLP1(1-37)	GLP-2	GLP(7-36)	Exendin-4
HillSlope	0.8982	0.9092	1.237	1.317
EC_{50}	4.887e-005	2.869e-009	1.564e-009	2.011e-009
S/B			15.0	26.0

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.

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





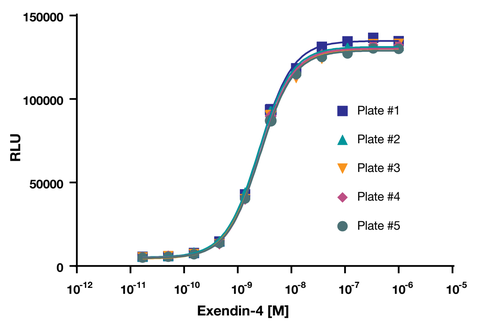
	Media	100% Plasma
HillSlope	1.039	0.6939
EC_{50}	1.570e-009	1.750e-009

	No Serum	1% Serum	2.5% Serum	5% Serum	10% Serum
HillSlope	0.9322	0.9889	1.018	1.043	0.9160
EC_{50}	2.781e-008	2.892e-008	2.423e-008	3.232e-008	3.457e-008
S/B	6.0	6.9	6.8	7.1	7.8

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*.

PathHunter Bioassays are Highly Reproducible

A



B

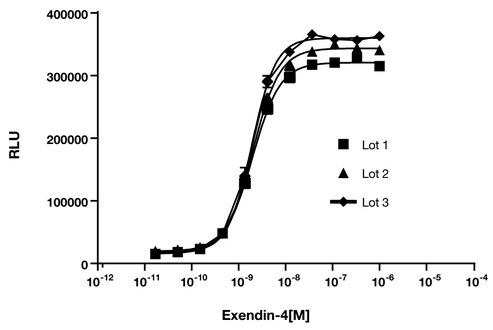


	Plate #1	Plate #2	Plate #3	Plate #4	Plate #5
HillSlope	1.393	1.395	1.365	1.423	1.396
EC_{50}	2.533e-009	2.426e-009	2.610e-009	2.616e-009	2.609e-009
S/B	27.2	25.3	29.7	26.4	27.5

	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 observe, the assay demonstrated a high reproducibility with 3-7 % CV.

Summary & Conclusions

- PathHunter cell-based bioassays are chemiluminescent assays, indicative of native receptor biology
- Assays give a target specific functional response
- Can tolerate up to 100% human serum and plasma
- PathHunter assays are specific, robust, and reproducible
- Simple and homogenous protocol with rapid results
- Thaw & Use kits with frozen ready-to-assay cells provide low variability between assay runs
- Suitable to identify neutralizing antibodies (NAbs) to biotherapeutic drugs in high concentrations of human serum
- Ability to function as both direct and indirect NAb assays
- World's largest menu of NAb assays

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