Accelerate Drug Development of GLP-1 & -2 Receptor Analogs with Ready-to-Use Cell Based Assays

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Abstract

There is an increasing need to generate therapeutics targeting metabolic and gastrointestinal diseases to address the global burden of metabolic diseases. By targeting G protein-coupled receptor (GPCRs) such as glucagon-like peptide (GLP-1 and GLP-2) receptors, therapeutics can be developed to address these globally prevalent medical disorders. In particular, engineered recombinant agonist analogs targeting the GLP-1 receptor are FDA approved for managing type-2 diabetes and body weight disorders, while GLP-2 analogs have been under therapeutic focus for the treatment of short bowel syndrome, a serious unmet medical disorder. Several other novel GLP analogs, including biosimilars are in clinical development; however, existing assays for characterizing their mechanism of action (MOAs) are complex and require extensive development time.

Eurofins DiscoverX® offers a comprehensive portfolio of fit-for-purpose products targeting GLP-1 and GLP-2 receptors. Based on the industry-validated Enzyme Fragment Complementation (EFC) technology, the GLP-1 and GLP-2 targeting products are offered with different molecular MOAs, such as cAMP accumulation or β-arrestin recruitment.

In this poster, we share example data of marketed therapeutics to qualify cell based potency assays (bioassays) targeting the GLP-1 and GLP-2 receptors. These ready-to-use bioassays are functional assays qualified with marketed drugs such as liraglutide, exenatide, and semaglutide for GLP-1R, and teduglutide for GLP-2R which reflect the MOA for the therapeutic action of these glucagon-like peptide analogs. We demonstrate that these bioassays are fit-for-purpose for implementation in quality control (QC) lot-release testing, helping to accelerate the time-to-market for therapeutic development by 6 to 9 months.

Significance of GLP-1 and GLP-2 in Metabolic Disorders

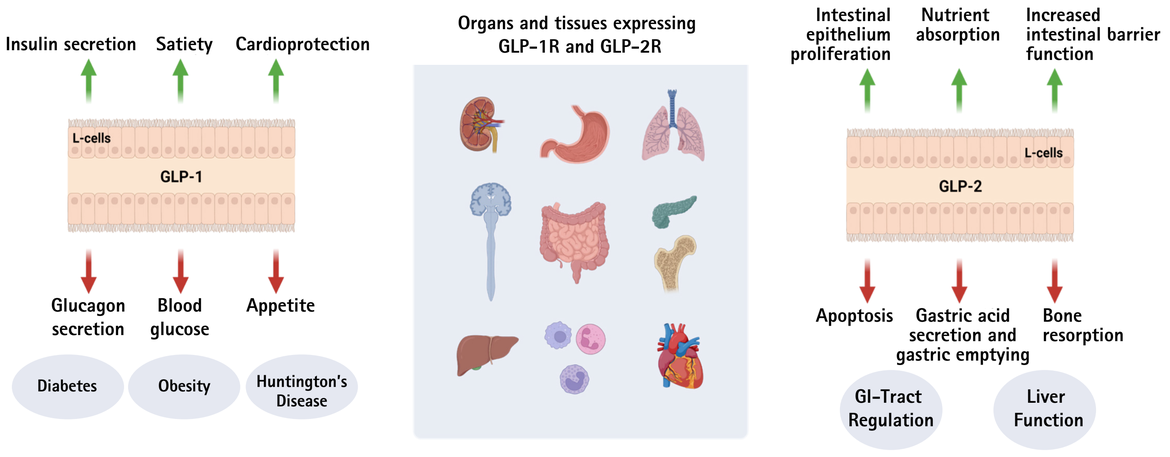
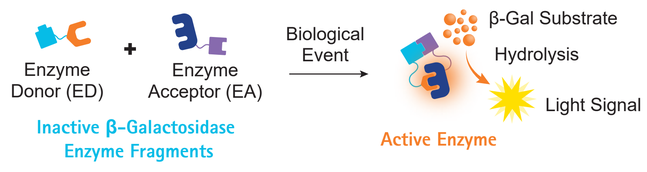


Figure 1. GLP-1 and GLP-2 mechanisms and organs expressing GLP-1R and GLP-2R. GLP-1 and GLP-2 are metabolically significant peptide hormones. Therapeutics targeting GLP-1R and GLP-2R are being developed to address globally prevalent medical disorders. Engineered recombinant agonist analogs targeting the GLP-1 receptor are FDA approved for managing type 2 diabetes, obesity, and body weight disorders. GLP-2 analogs have been under therapeutic focus for the treatment of short bowel syndrome, a serious unmet medical disorder.

Functional, Cell-based Assays for Glucose Metabolism Targets for Multiple MOAs

A **Enzyme Fragment Complementation (EFC)**



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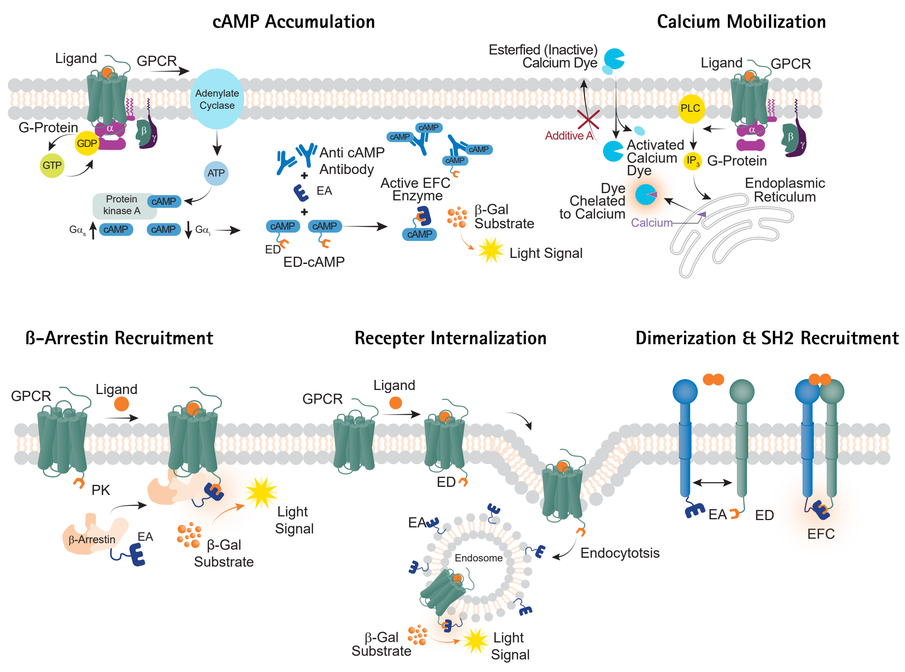
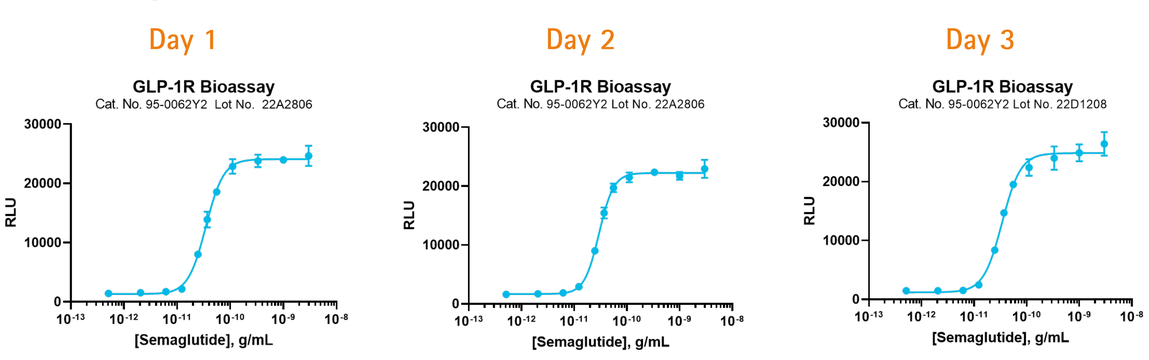


Figure 2. Functional, cell-based assay formats for GPCRs and RTKs involved in glucose metabolism. A. The proprietary Eurofins DiscoverX EFC technology is based on a split β-galactosidase (β-gal) enzyme. Enzyme activity is measured with addition of detection reagent containing luminescent enzyme substrate and detecting the complementation of the ED and EA fragments. B. A variety of functional assay formats for drug targets involved in regulation of glucose metabolism including cell lines and bioassays that evaluate multiple drug MOAs for important targets in the glucagon receptor family, including GLP-1R, GLP-2R, GIPR, and GCGR. Assays are available to evaluate activation of second messenger pathways (e.g. cAMP and Ca^{2+}), β-arrestin recruitment, receptor internalization, and RTK (insulin receptor) dimerization, phosphorylation, and SH2 recruitment.

Qualified Semaglutide (GLP-1R) Bioassay Exhibits Excellent Repeatability Across Experiments



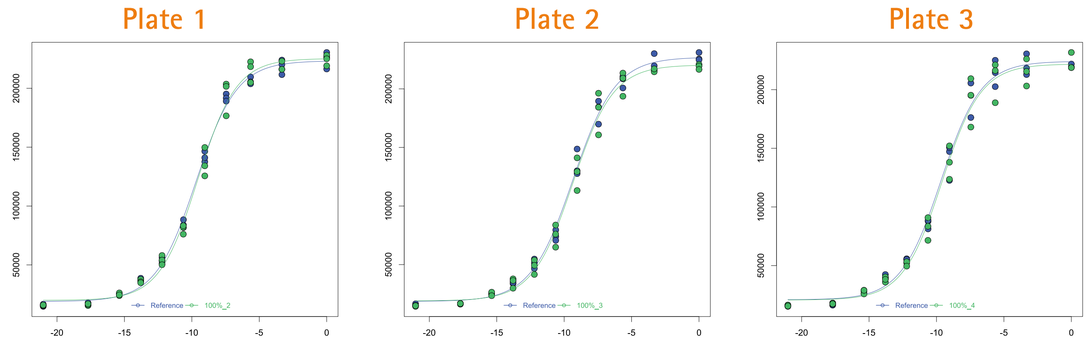
	Day 1	Day 2	Day 3	% RSD
S/B	15.8	13.9	16.3	8.3%
EC_{50}, pg/mL	34.8	29.7	34.1	8.4%

Figure 3. Excellent consistency of dose response for semaglutide (GLP-1R) bioassay. Evaluation of repeatability of response to semaglutide (Ozempic®) was performed using the cAMP Hunter™ Semaglutide Bioassay that measures cAMP production in response to semaglutide treatment. The experiment was performed on 3 different days by a single analyst with an optimized dose curve of semaglutide used to qualify the bioassay. Data from day 1 and 2 were generated with the same lot of CHO-K1 GLP1R bioassay cells, while day 3 data used a different lot. These data demonstrate the excellent consistency in response with respect to absolute S/B (signal-to-background), EC_{50} and RLU, even across different lots of cells.

Ozempic is a registered trademark of Novo Nordisk A/S. Victoza is a registered trademark of Novo Nordisk A/S. Byetta is a registered trademark of AstraZeneca. Gattex is a registered trademark of Shire-NPS Pharmaceuticals, Inc.

Relative Potency Data Using the GLP-2R Bioassay with Teduglutide

A



B

Sample	Max % CV	S/B	Relative Potency Ratio	95% Confidence Interval	Relative Confidence Interval (% Relative CI)	F-test for Linearity / F-test for Parallelism
Plate 1 (100%)	9%	11.3	0.95805	0.85332 - 1.07564	89.07% - 112.27% (23.21%)	Failed / Passed
Plate 2 (100%)	12.8%	11.6	0.92213	0.79886 - 1.06442	86.63% - 115.43% (28.80%)	Passed / Passed
Plate 3 (100%)	11.9%	10.8	0.91961	0.75783 - 1.11594	82.41% - 121.35% (38.94%)	Passed / Passed

Figure 4. Relative potency data for PathHunter® GLP-2R Bioassay using teduglutide. A. Preliminary relative potency data for 100% nominal concentration (NC) of Gattex® (teduglutide) in the PathHunter GLP-2R Bioassay. Three independent assessments of teduglutide (plates 1-3) were performed using the recommended assay conditions. Data shown are the restricted model (common slope and asymptotes) from PLA 3.0 analysis software (Stegmann Systems) based on a 95% confidence interval around the 4 parameter logistic (4PL) fit model for the 100% nominal potency of teduglutide. In each graph, the dark blue curve represents the reference standard, while light green corresponds to the 100% test sample. Relative potency (represented as %) calculated by PLA 3.0 is shown with each graph. B. Statistics calculated by PLA 3.0. Last column shows whether the two curves in the restricted model pass F-tests for linearity and parallelism. While further optimization is still underway, these data suggest this assay should be suitable for development of a fit-for-purpose assay to assess relative potency of teduglutide.

Qualification Data for Semaglutide (GLP-1R) Bioassay Kit Demonstrating Suitability for Relative Potency Applications

A **Qualification Study Design**

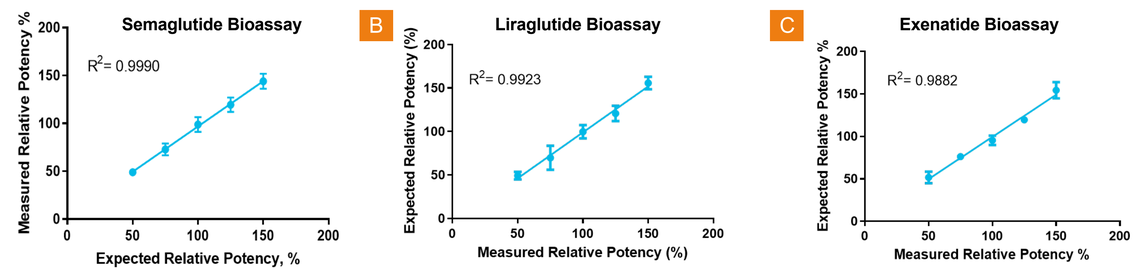
- Evaluated 5 nominal concentrations (NC) over a range of 50% to 150%
  - (n ≥ 4 for each NC)
- Repeatability: 4 runs of 100% NC by single analyst
- Intermediate precision incorporated:
  - 2 Analysts
  - Multiple days
  - 2 Lots of bioassay cells

B **Qualification Study Results**

Nominal RP, %	Analyst	Observed RP, %	Average RP, %	% RSD	Average % Recovery
150	1	149.1	143.9	5.4	95.9%
	1	138.8			
	1	135.8			
	2	151.9			
`125	1	130.7	119.4	6.4	95.5%
	1	116.7			
	1	115.2			
	2	114.8			
100	1	108	98.8	7.8	98.8%
	1	101.1			
	1	88.1			
	1	94.4			
	2	102.3			
75	1	73.5	72.8	8.5	97.1%
	1	79.0			
	1	64.3			
	2	74.4			
50	1	47.3	48.9	3.9	97.9%
	1	51.1			
	1	50			
	2	47.3			

Figure 5. Qualification data for the semaglutide (GLP-1R) bioassay kit. A. Assay qualification study design. Five nominal concentrations (NCs) of semaglutide over a range of 50% to 150% were evaluated (n ≥ 4 for each NC). Repeatability was evaluated using the 100% NC run by a single analyst in one day. Intermediate precision incorporated 2 analysts, multiple days, and evaluation of 2 different lots of bioassay cells. For this study, no plates failed pre-established system and sample suitability criteria for the assay. B. Qualification study results, also summarized in table in Figure 6. D., demonstrating good accuracy, repeatability, intermediate precision, and dilutional linearity for the semaglutide bioassay kit.

Broad Portfolio of GLP-1R Bioassays Qualified with Multiple Innovator Drugs for Relative Potency Applications



D

Parameter	Semaglutide (Oxempic)	Liraglutide (Victoza)	Exenatide (Byetta)	Specification
Accuracy (Average % Recovery)	97%	97.9%	101%	100% +/- 20%
Repeatability	8.7%	7.7%	5.7%	≤20%
Intermediate Precision	≤8.5%	≤20%	≤9.6%	≤20%
Linearity (R^2)	0.9990	0.9923	0.9882	≥0.95

Figure 6. Summary of the assay performance of the bioassay kit (GLP-1R) with innovator drugs. The bioassay GLP-1R bioassay kit was qualified with innovator drugs, Ozempic (semaglutide), Victoza® (liraglutide), and Byetta® (exenatide), over a range of 50% to 150%, using two analysts. A., B., and C. Dilutional linearity was plotted using data from the 5 NCs tested (50%, 75%, 100%, 125%, and 150%) in each study. D. Results of each qualification study showing overall accuracy, repeatability (based on testing of the 100% NC), intermediate precision, and dilutional linearity for each bioassay, along with the specification for each parameter. All three assays met the specifications established for a successful, fit-for-purpose assay for relative potency applications.

Summary

- Eurofins DiscoverX offers a portfolio of GLP-1 and -2 cell lines and ready-to-use bioassays for characterization through QC lot release. These cell-based MOA-reflective, functional assays are robust and sensitive.
- Evaluate both targets (GLP-1R and GLP-2R) for a comprehensive understanding of therapeutic MOAs with different readouts such as cAMP accumulation, β-arrestin recruitment, and receptor internalization
- Accelerate your drug release program into QC lot release with bioassays qualified with approved therapeutics like Ozempic (semaglutide), Victoza (liraglutide), and Byetta (exenatide)
- Eurofins DiscoverX GLP-1 and GLP-2 qualified bioassays have been validated at CRO's
- Development of a qualified bioassay with the innovator drug Gattex (teduglutide) is in progress, but preliminary relative potency data suggests the PathHunter GLP-2R Bioassay may be suitable for development of a fit-for-purpose method to assess relative potency of teduglutide
- IND-enabling characterization and assay qualification studies with the clinical molecules can be performed through Eurofins DiscoverX custom development program

Learn more about Eurofins DiscoverX's products and custom development capabilities at discoverx.com