Qualified, Fit-for-Purpose Bioassays for Liraglutide and Exenatide as Frozen Ready-to-Use Cells

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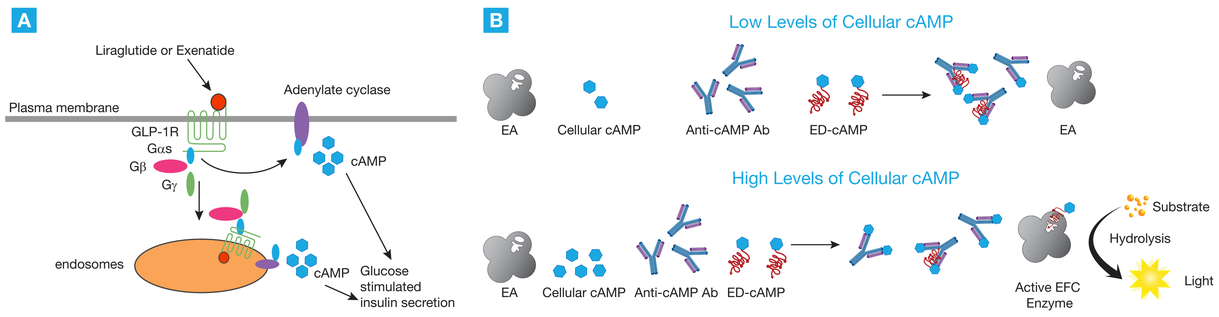
Abstract

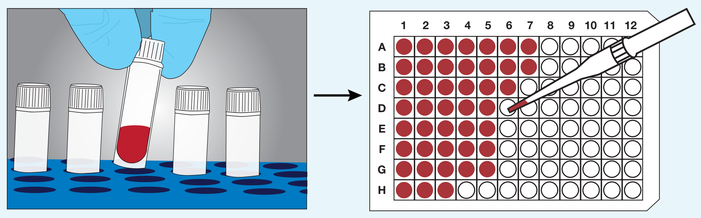
With more than 25% of the world's population diagnosed with type II diabetes and/or metabolic syndrome, the requirement for cost-effective drugs to control patient disease is increasingly urgent. Agonist drugs of the Glucagon-like peptide-1 (GLP-1) receptor, such as Victoza® (Liraglutide) and Byetta® (Exenatide), modulate glucose-induced insulin secretion and have shown excellent results in the clinic. These molecules are highly sought after for biosimilar and biobetter development, however, the existing cell-based assays to support this development are complex and require extensive development time. Here, we describe the development and qualification of ready-to-use (RTU), fit-for-purpose potency bioassays for these two drugs. Based on the drug's well-characterized mechanism of action, these bioassays measure production of cyclic AMP (cAMP) in response to activation of the GLP1 receptor with agonist drug. The assay relies on an easy-to-use homogenous protocol for rapid implementation at any lab globally, resulting in a chemiluminescent readout that can be read on any plate reader. The assay was qualified using the marketed innovator molecules through a multi-day qualification exercise with two analysts. The assays demonstrated high reproducibility, accuracy, and precision, with good linearity over the tested range of 50%-150%. Importantly, these assays have been developed into frozen RTU cells and the data presented here demonstrates the multiple technical and operational advantages of this approach over the traditional continuous culture assays.

Measure Cellular cAMP After GLP-1 Agonist Activation



A. Natural biology of GLP-1 receptor activation and its contribution to glucose-stimulated insulin secretion (GSIS) in cultured cells. GLP-1R is activated by liraglutide or exenatide and its activation generates cellular cAMP at the membrane and also at endosomes, both of which contribute to GSIS. (Figure adapted from American Journal of Physiology - Endocrinology and Metabolism, 2013.) B. The cAMP detection kits are based on competitive immunoassays using a split β-galactosidase (β-gal) enzyme system. The enzyme donor (ED) is conjugated with cAMP and this ED-cAMP conjugate and cellular cAMP compete for binding to an anti-cAMP antibody (Ab). With low levels of cellular cAMP, the ED-cAMP outcompetes the cellular cAMP for binding to the Ab, making the ED-cAMP unable to complement with the enzyme acceptor (EA). With high levels of cellular cAMP, the anti-cAMP antibody becomes saturated allowing the ED-cAMP to complement with the EA and form an active β-gal enzyme. The active enzyme hydrolyzes a substrate to produce a chemiluminescent signal that is directly proportional to the level of cAMP in the cells.

Use of Cryopreserved Ready-to-Use Cells

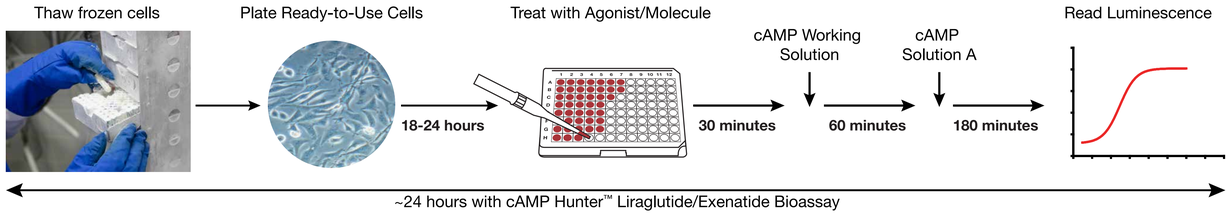


Frozen Ready-to-Use Cells

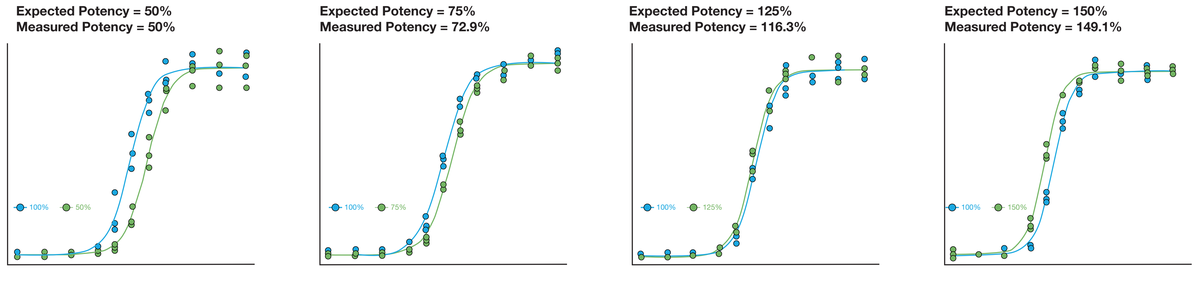
- Higher efficiency
- Greater assay reproducibility
- Operational flexibility
- Cost savings - Serum, culture media, and reagents
- Ease of assay development and method transfer to global sites

DiscoverX bioassay cells, manufactured and included as part of bioassay kits, are meant for single use in ready-to-use vials. The frozen cells are taken from their cryopreserved storage, thawed, and plated directly onto plates to run the assay. This format has several advantages including higher efficiency as fewer resources are needed for cell maintenance and media preparation, more consistent assay performance as all the cells in a bank are frozen at the same passage number, and greater operational flexibility as assays can be performed as and when needed. Together, these lead to significant cost and time savings.

Easy-to-Use Protocol with Results in 24 Hours



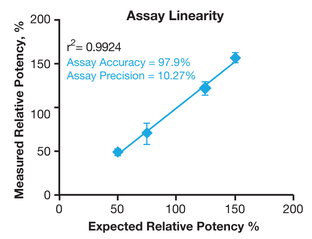
Qualification of Liraglutide Bioassay with Victoza®



Relative potency of Liraglutide (Victoza®) was quantified using the cAMP Hunter GLP-1R bioassay cells in a frozen RTU format, over a range of concentrations (50%-150%), relative to a 100% reference standard. Data is shown for a single analyst as plotted in PLA using a 4PL fit (top). As shown in the panels above, the upper and lower asymptotes of the reference standard and the sample curve are both fully defined, and the samples are parallel to the reference standard, enabling accurate relative potency measurements to be reported. All calculated relative potencies are shown in the table on the next panel. Victoza® is a registered trademark of Novo Nordisk.

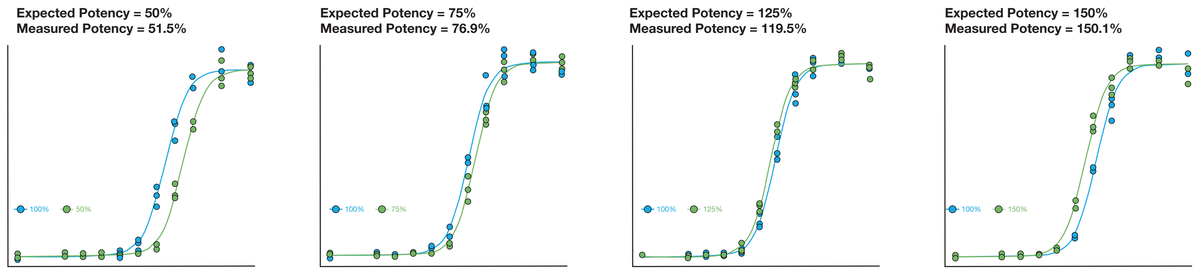
cAMP Hunter Liraglutide Bioassay Is Fit-for-Purpose & QC-Suitable

Analyst	Day	Expected Potency, %	Measured Potency, %	Mean Potency, %	SD, %	Recovery, %	RSD, %
Analyst 1	1	50	42.7	49.18	4.46	98	9.06
Analyst 2	2		50				
	3		52.7				
	4		51.3				
Analyst 1	1	75	57	69.70	13.95	93	20.02
Analyst 2	2		88				
	3		60.9				
	4		72.9				
Analyst 1	1	125	118.8	120.83	8.98	97	7.43
Analyst 2	2		134				
	3		114.2				
	4		116.3				
Analyst 1	1	150	155.9	155.73	7.15	104	4.59
Analyst 2	2		165.6				
	3		149.1				
	4		152.3				



To demonstrate that the assay is fit-for-purpose, the assay was qualified using Victoza® with two analysts over 3 days over a range of concentrations relative to a 100% reference standard. The cells used here were frozen RTU cells from DiscoverX and all the data was in PLA using a 4PL fit (table on the left). The average measured relative potency was plotted against the expected relative potency (graph). The assay linearity graph quantifies data generated by 2 analysts using this bioassay over four days.

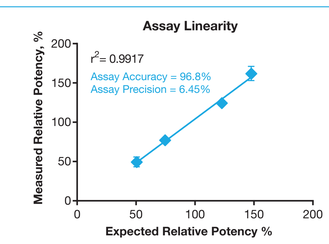
Qualification of Exenatide Bioassay with Byetta®



Relative potency of Exenatide (Byetta®) was quantified using the cAMP GLP-1R bioassay cells in a frozen RTU format, over a range of concentrations (50%-150%), relative to a 100% reference standard. Data is shown for a single analyst as plotted in PLA using a 4PL fit (top). As shown in the panels above, the upper and lower asymptotes of the reference standard and the sample curve are both fully defined, and the samples are parallel to the reference standard, enabling accurate relative potency measurements to be reported. All calculated relative potencies are shown in the table below. Byetta® is a registered trademark of Eli Lilly and Company.

cAMP Hunter Exenatide Bioassay Is Fit-for-Purpose & QC-Suitable

Analyst	Day	Expected Potency, %	Measured Potency, %	Mean Potency, %	SD, %	Recovery, %	RSD, %
Analyst 1	1	50	61.5	51.83	6.90	104	13.32
Analyst 2	2		48.8				
	3		45.5				
	4		51.5				
Analyst 1	1	75	78.6	76.28	3.20	102	4.19
Analyst 2	2		76.9				
	3		71.6				
	4		78				
Analyst 1	1	125	119.3	119.55	2.54	96	2.12
Analyst 2	2		119.5				
	3		122.8				
	4		116.6				
Analyst 1	1	150	151.4	154.38	9.56	103	6.19
Analyst 2	2		147.5				
	3		168.5				
	4		150.1				



To demonstrate that the assay is fit-for-purpose, the assay was qualified using Byetta® with two analysts over 3 days over a range of concentrations relative to a 100% reference standard. The cells used here were frozen RTU cells from DiscoverX and all the data was in PLA using a 4PL fit. (table on left) The average measured relative potency was plotted against the expected relative potency (graph). The assay linearity graph quantifies data generated by 2 analysts using this bioassay over four days.

High Reproducibility of Exenatide Bioassay

Assay Robustness (EC_{50})	Exenatide, %RSD	Liraglutide, %RSD
Intra-Assay Precision (repeatability)	4.1	8.6
Intra-Day Precision (intermediate precision)	9	11
Inter-Day Precision (intermediate precision)	18.8	12.1

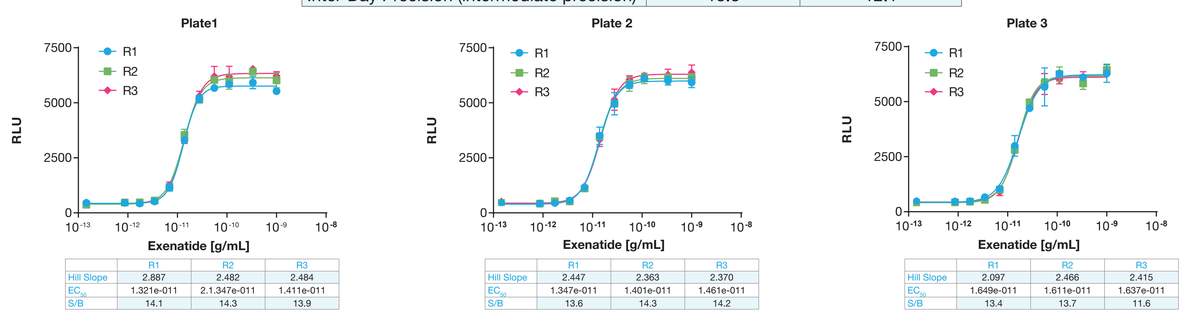


	Plate1 R1	Plate1 R2	Plate1 R3
Hill Slope	2.887	2.482	2.484
EC_{50}	1.321e-011	2.1.347e-011	1.411e-011
S/B	14.1	14.3	13.9

	Plate 2 R1	Plate 2 R2	Plate 2 R3
Hill Slope	2.447	2.363	2.370
EC_{50}	1.347e-011	1.401e-011	1.461e-011
S/B	13.6	14.3	14.2

	Plate 3 R1	Plate 3 R2	Plate 3 R3
Hill Slope	2.097	2.466	2.415
EC_{50}	1.649e-011	1.611e-011	1.637e-011
S/B	13.4	13.7	11.6

To assess the reproducibility of the bioassay using frozen RTU cells, the assay was performed on 3 different plates over the course of three days with a single analyst. The cAMP Hunter GLP-1R bioassay cells were seeded into 3 x 96-well plates and three experimental replicates of Exenatide were prepared in dose response format. Each experimental replicate was run in duplicate using the optimized protocol. Assay robustness metrics, based on EC_{50}, are shown in the table above as intra-assay precision (% RSD; n=3 samples/plate) and intermediate precision [broken out as intra-day precision (plate-to-plate; n=3 plates) and inter-day precision (n=3 days for exenatide, 4 days for liraglutide)].

Summary

Fit-for-Purpose MOA-Based Bioassays for Liraglutide and Exenatide

- MOA-based bioassay with frozen RTU cells expressing human GLP-1R and a homogenous, no-wash cAMP detection method that generates results in 24-26 hours, with an easy-to-use protocol
- Data demonstrates highly accurate and precise measures of relative potency over a linear range of 50% to 150% of reference standard over multiple days, with multiple analysts
- The assay and method are deemed fit for the purposes of evaluating the functional comparability and relative potency of liraglutide & exenatide biosimilar and innovator materials

Benefits of cAMP Hunter Liraglutide and Exenatide Bioassay Kits

- Available frozen ready-to-use cells for rapid assay development & transfer without any cell culture necessary
- Easy-to-use protocol enabling quick implementation in any lab & with any analyst
- Highly reproducible data reducing the number of failed tests
- Saves months of assay development time translating into overall cost savings in a biosimilar development program

Go to discoverx.com/biosimilars to see additional assays

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