DiscoverX

Optimizing and Qualifying Bioassays for Biosimilars, Biobetters, and Innovator Drugs

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DiscoverX

Global Leader in Cell-Based Assays for Drug Discovery

7+ Druggable target classes

900+
Stable cell lines

20+
core patents

Reliable
> 30 Billion data points screened with EFC technology

15+
years of experience

Addressing unmet market needs

Clear value proposition

2000+
Assay ready kits

Validated 15+ Years
Partnering experience with Industry leaders

1761+
Publications across multiple applications

Enabling assays for any drug discovery program

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Bioassay Requirements for QC

Industry considerations

- Must be reflective of the MOA of the molecule
- Must be suitable for use in QC
  - Robust, precise, accurate and linear (preferably over a range of 50%-150%)
  - Protocol should enable facile transfer to multiple sites and easy adoption using equipment readily available in most CRO labs
  - Amenable to high throughput and low cost
- Must be sensitive to structural changes in the molecule and stability indicating

DiscoverX Optimization and Qualification Workflow

Optimization & qualification of bioassays with innovator molecule, when available

Assay Selection

Choose assay format that best reflects MOA of drug

Assay Optimization

Optimize assay parameters

Testing Innovator Drug

Refine conditions with innovator drug

Assay Qualification

Establish robustness, precision, accuracy, and linearity of assay

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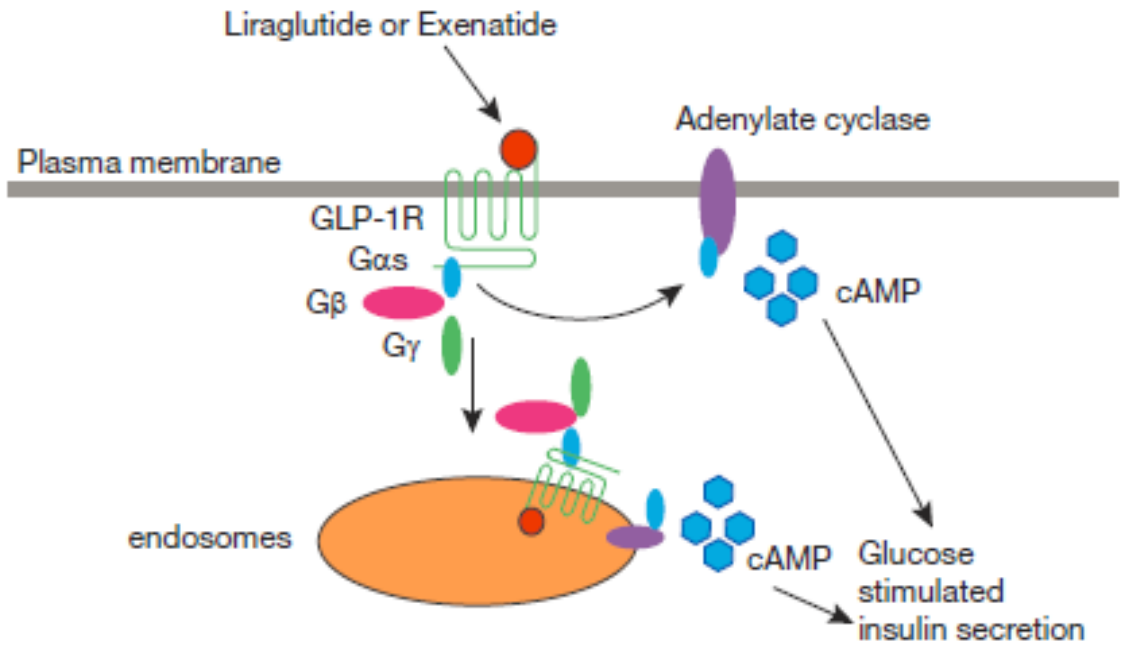
Case Study: GLP1R Agonist Drugs

Drug's mechanism of action



- GLP1 analogs improve blood glucose levels in adults with type 2 diabetes
- GLP1R agonist binding leads to receptor activation, resulting in increased cAMP production, ultimately leading to elevated insulin secretion

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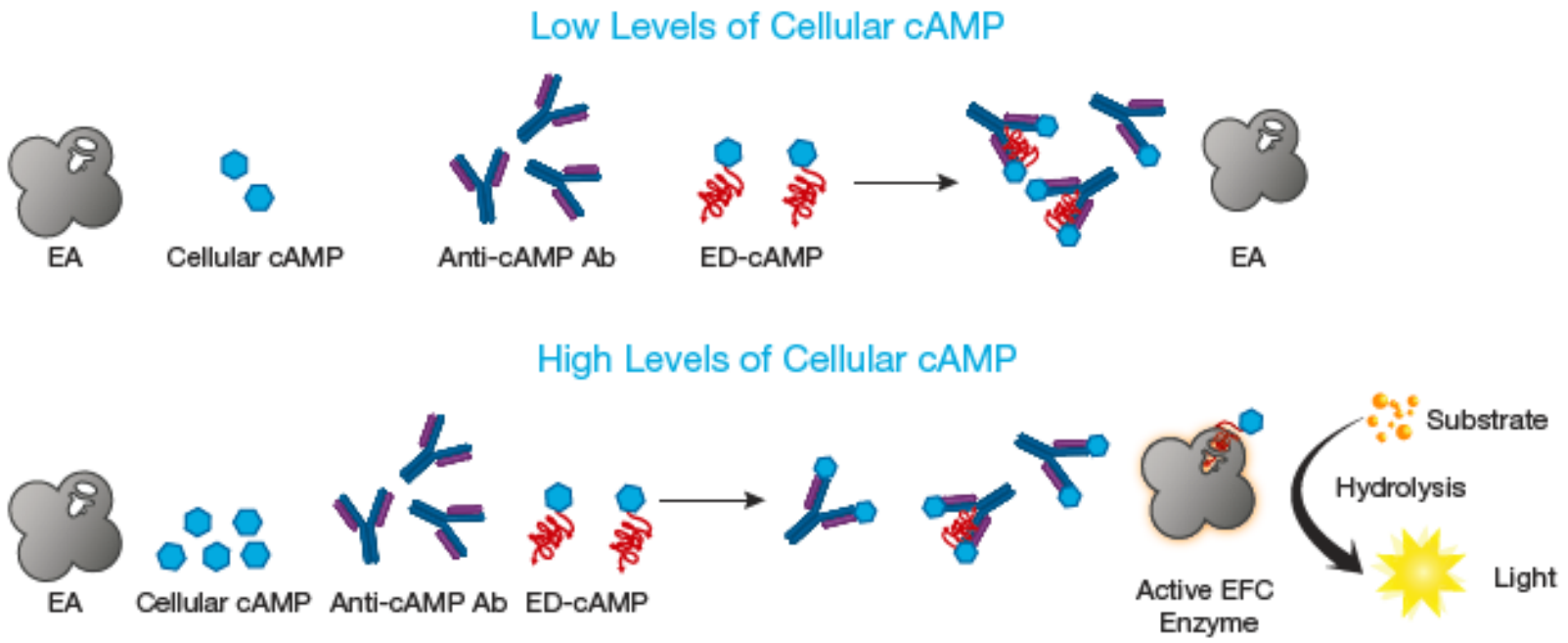
Bioassay Principle for GLP1R Agonists

cAMP detection through Enzyme Fragment Complementation



- ED-cAMP competes with endogenously produced cAMP for binding to anti-cAMP Ab
- High cAMP levels leads to increased β-gal complementation and increased luminescence

6
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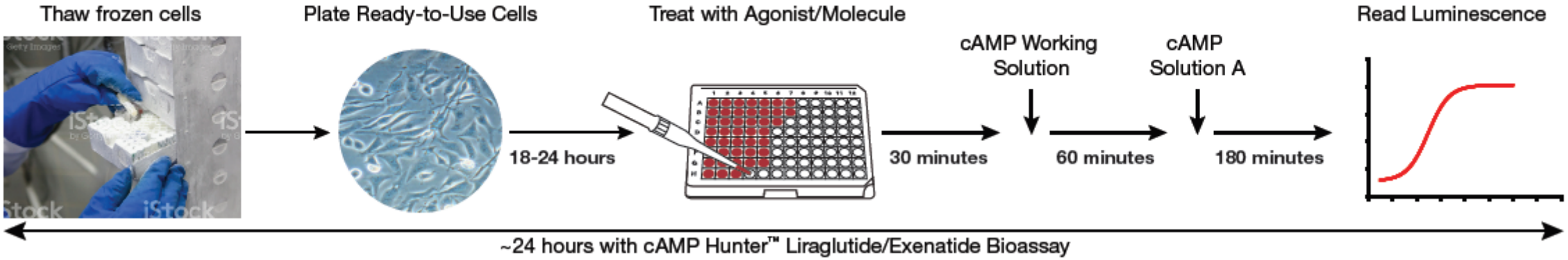
Easy-to-Use Protocol with Results in 24 Hours



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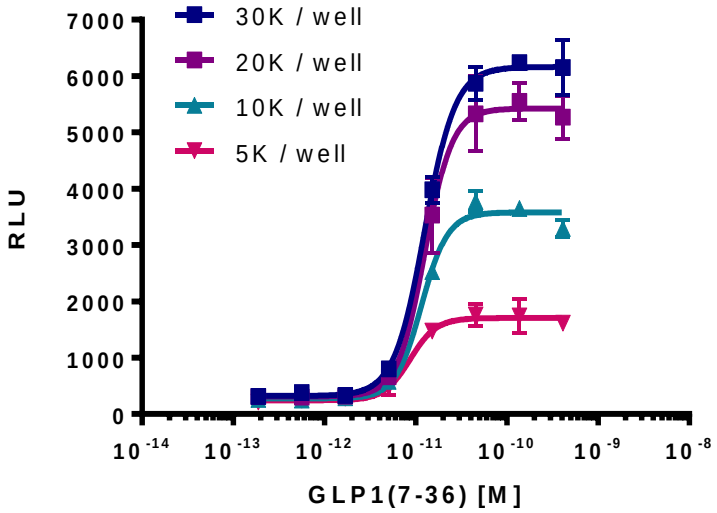
GLP1R Assay Optimization

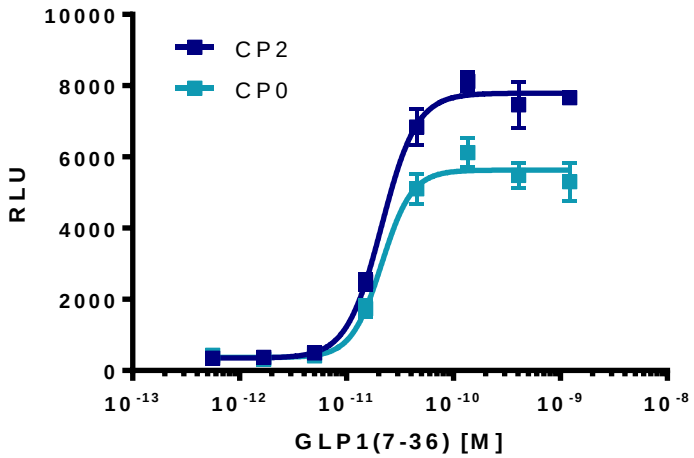
Examples of variables evaluated for frozen ready-to-use cells

Cell Density Optimization



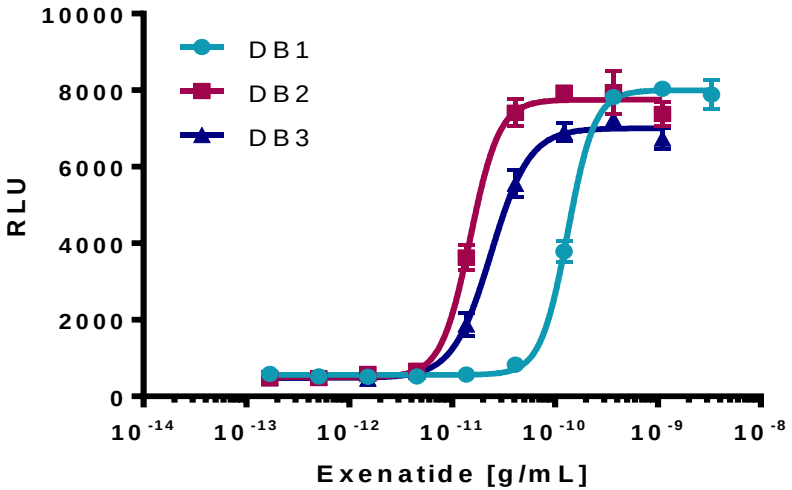
	30K/well	20K/well	10K/well	5K/well
EC_{50}	1.23×10^{-11}	1.27×10^{-11}	1.1×10^{-11}	7.5×10^{-12}
S/B	14.8	15.0	11.7	5.0

Plating Reagent



	CP2	CP0
EC_{50}	2.14×10^{-11}	2.13×10^{-11}
S/B	19.6	11.5

Dilution Buffer



	DB1	DB2	DB3
EC_{50}	1.33×10^{-10}	1.5×10^{-11}	2.39×10^{-11}
S/B	13.6	15.5	14.0

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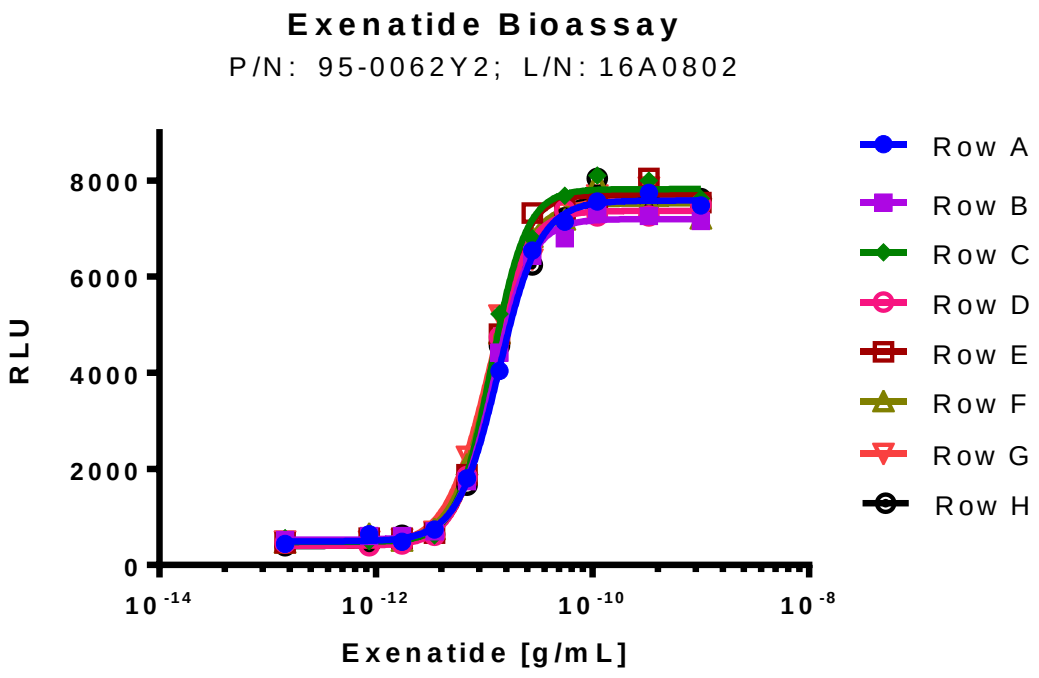
Evaluation of Assay Robustness & Edge Effects

Excellent reproducibility with modest edge effects



All Rows Included

Rows	R^2	S/B	Mean EC_{50}	SD	% RSD
A and B	0.999	13.9	$1.3x10^{-11}$	$9.2x10^{-13}$	7.1
C and D	0.998	14.3	$1.16x10^{-11}$	$1.2x10^{-13}$	1.0
E and F	0.997	16.1	$1.17x10^{-11}$	$2.7x10^{-13}$	2.3
G and H	0.996	15.1	$1.21x10^{-11}$	$1.5x10^{-12}$	12.8

Outer Rows Excluded

Rows	R^2	S/B	Mean EC_{50}	SD	% RSD
B and C	0.998	14.0	$1.2x10^{-11}$	$4.4x10^{-13}$	3.6
D and E	0.999	15.5	$1.17x10^{-11}$	$2.3x10^{-13}$	2.0
F and G	0.996	14.6	$1.12x10^{-11}$	$3.8x10^{-13}$	3.4

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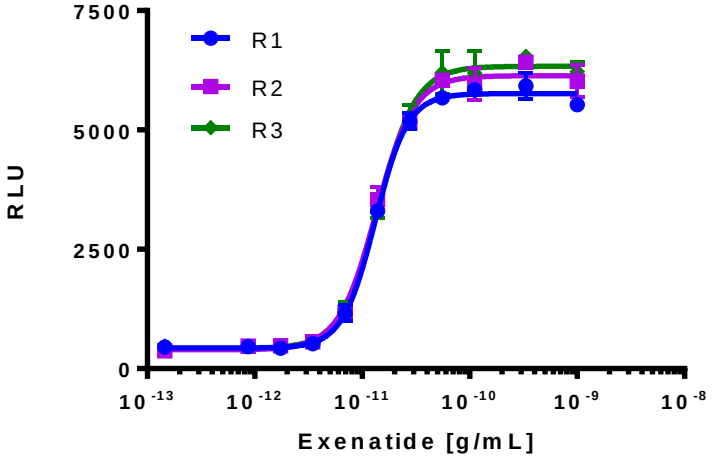
Intermediate Precision of Bioassay

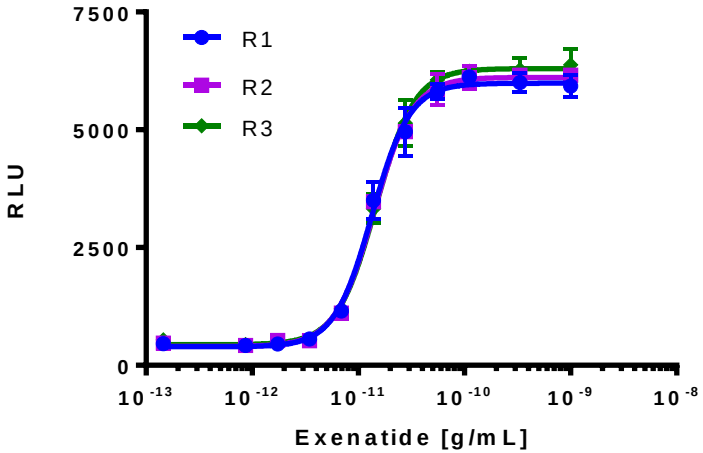
Inter-plate and inter-day data with Exenatide and Liraglutide

Plate 1



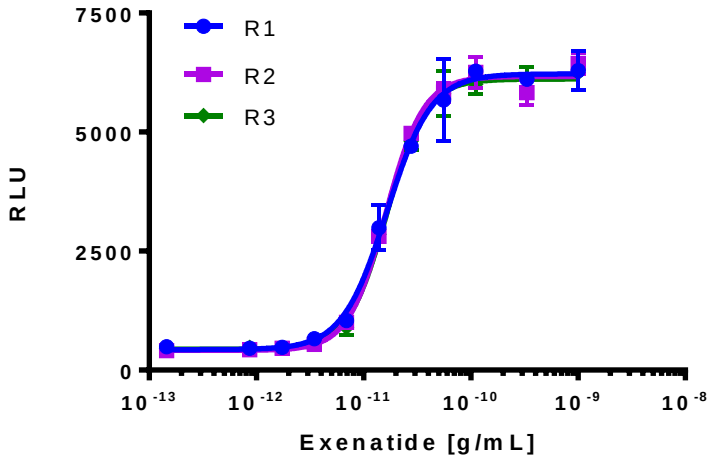
	R1	R2	R3
HillSlope	2.867	2.482	2.464
EC50	1.321e-011	1.347e-011	1.411e-011
S:B	14.1	14.3	13.9

Plate 2



	R1	R2	R3
HillSlope	2.447	2.363	2.370
EC50	1.347e-011	1.401e-011	1.461e-011
S:B	13.6	14.3	14.2

Plate 3



	R1	R2	R3
HillSlope	2.097	2.466	2.415
EC50	1.649e-011	1.611e-011	1.637e-011
S:B	13.4	13.7	11.6

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

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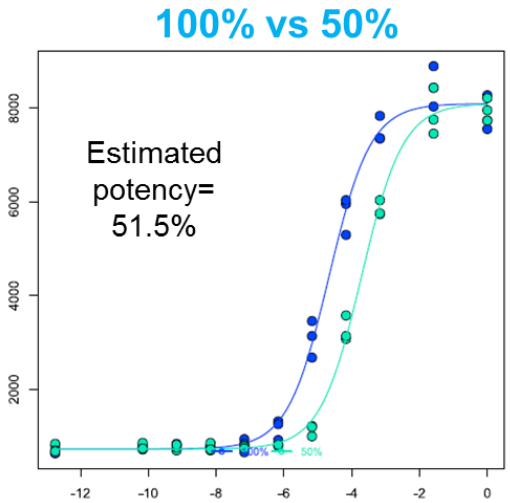
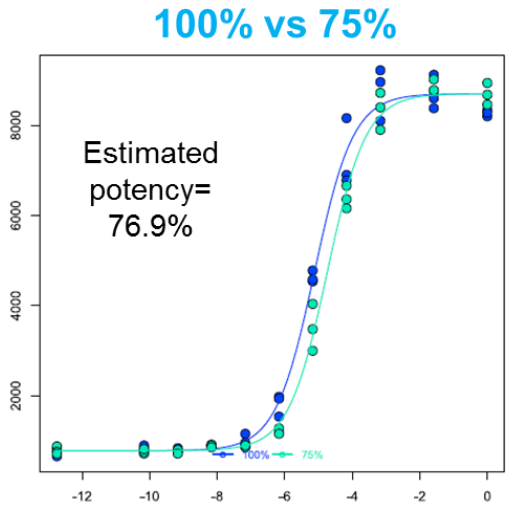
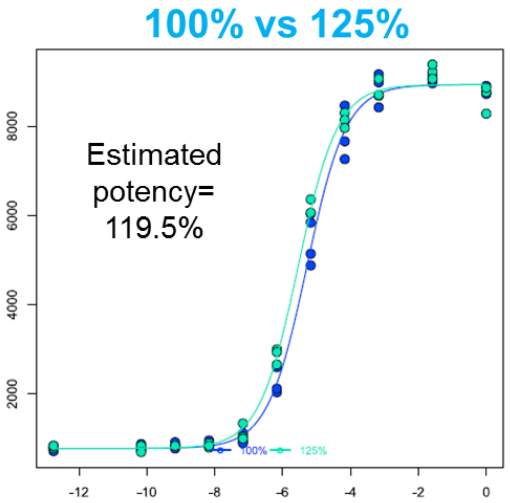
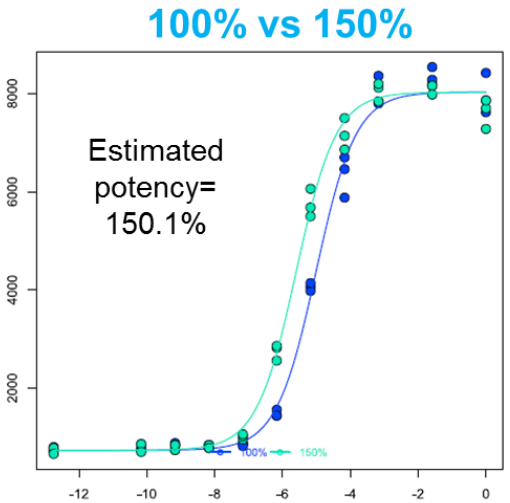
Estimation of Relative Potency

Parallel lines with tight confidence intervals for relative potency









Expected Potency (%)	Estimated Potency (%)	95% Confidence Interval (%)
50	51.4	47.28- 55.99 (16.93%)
75	76.9	70.35- 84.0 (17.87%)
125	119.5	112.3- 127.0 (12.31%)
150	150.1	140.7- 160.12 (12.92%)

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Assay Qualification with a Single Analyst (Liraglutide)

Good accuracy, precision, and linearity of assay

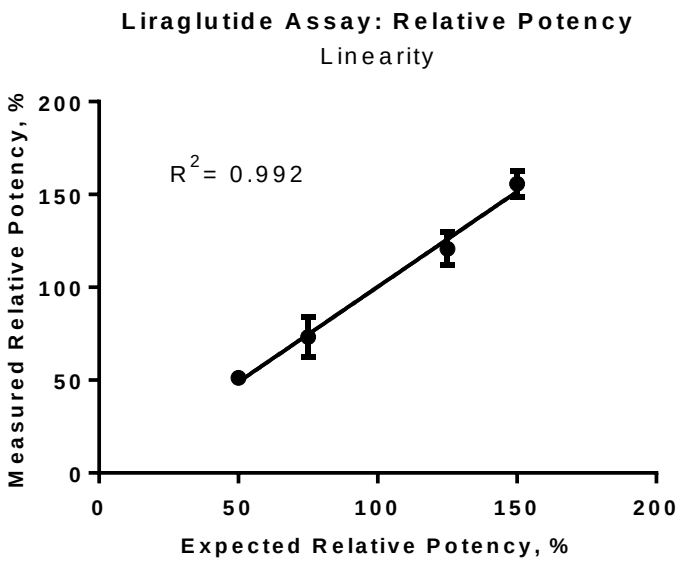
Expected RP	Measured RP		Average RP	% Recovery	SD	% RSD
150	165.6	149.1	155.7	103.8%	7.1	4.59
	152.3	155.9				
125	116.3	114.2	120.8	96.7%	9.0	7.43
	134	118.8				
75	88	60.9	73.3	97.8%	11.1	15.21
	72.9	71.5				
50	50	52.7	51.3	102.7%	1.1	2.15
	51.3	51.3				
			Accuracy	**97.0%**	**Precision**	**7.35%**

All samples passed F-test for parallelism in PLA software

Accuracy, % : 97.0

Precision, % : 7.4

Linearity, R^2: 0.992



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Assay Qualification with Two Analysts (Exenatide)

Good correlation between analysts performing the same assay on different days

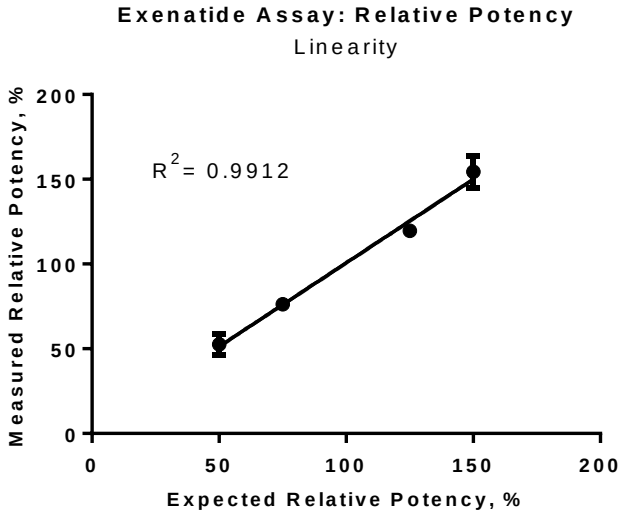
Expected RP	Measured RP (Analyst 1)		Measured RP (Analyst 2)	Average RP	% Recovery	SD	% RSD
150	147.5	150.1	151.4	154.4	102.9%	9.56	6.19
	168.5						
125	119.5	122.8	119.3	119.6	95.6%	2.54	2.12
	116.6						
75	76.9	78	78.6	76.3	101.7%	4.0	4.19
	71.6						
50	48.8	48.5	61.5	52.6	105.2%	6.1	11.6
	51.5						
				Accuracy	**96.5%**	**Precision**	**6.03%**

All samples passed F-test for parallelism in PLA software

Accuracy, % : 96.5

Precision, % : 6.03

Linearity, R^2: 0.9912



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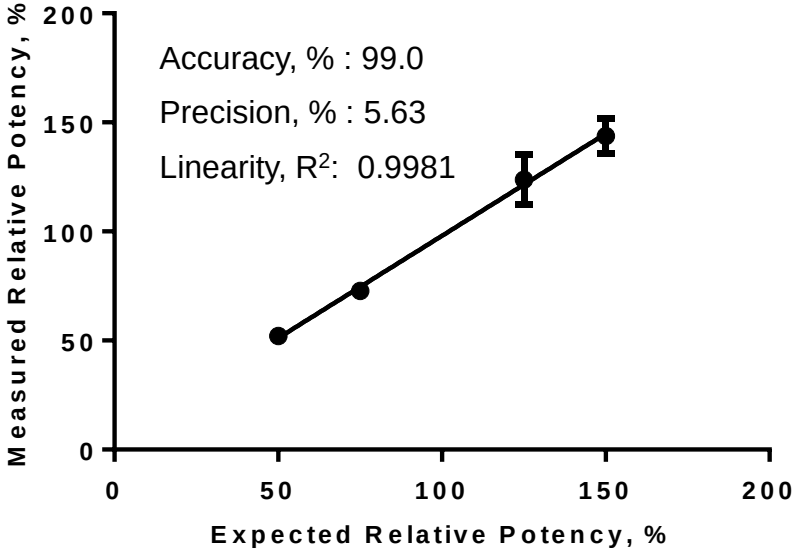
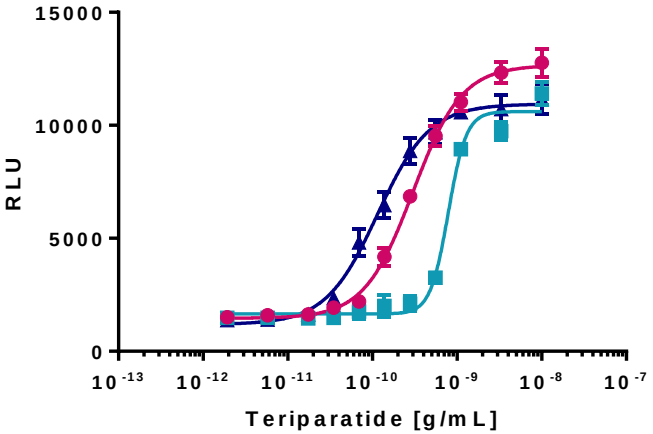
Assay Qualification for Teriparatide (Forteo®)

Excellent accuracy, precision, linearity, and stability indicating



Testing Heat-denatured Samples



	HillSlope	EC50
▲ Reference	1.416	1.151e-010
● 1hr @ 65C	1.510	3.015e-010
■ 2hr @65C	4.083	7.881e-010

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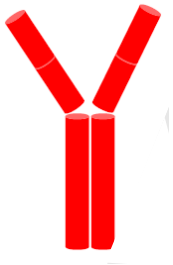
PathHunter® Bioassay for Anti-VEGF Therapeutics

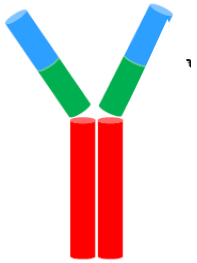
Functional assays based on native receptor biology

Bevacizumab (Avastin®)



- Humanized IgG monoclonal Ab
- Binds all VEGF-A isoforms

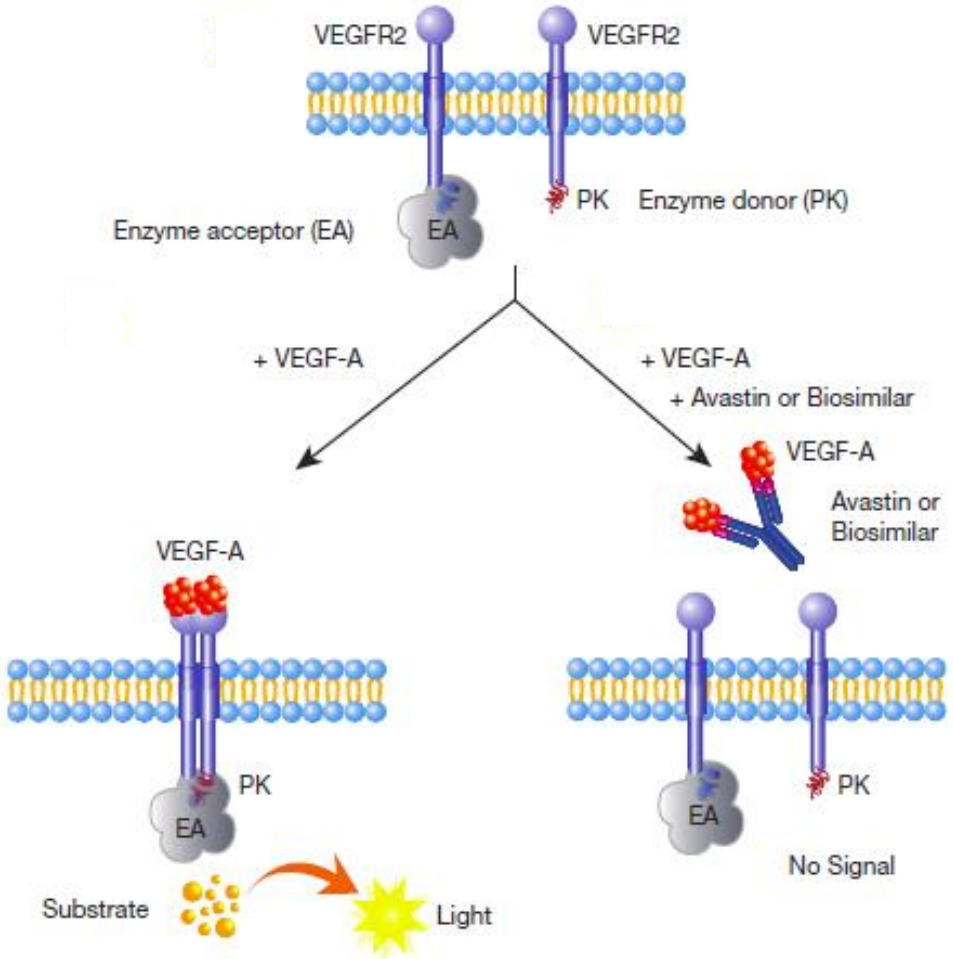
Aflibercept (Eylea®)



- Fusion Protein; ECDs of VEGFR1 and VEGFR2
- Binds all VEGF-A, B isoforms, PlGF

Ranibizumab (Lucentis®)

- Fab fragment
- Binds all VEGF-A isoforms



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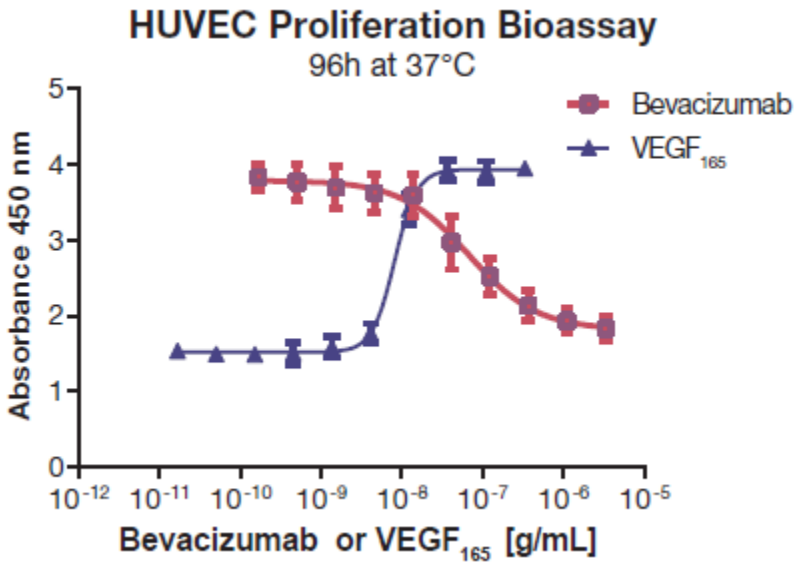
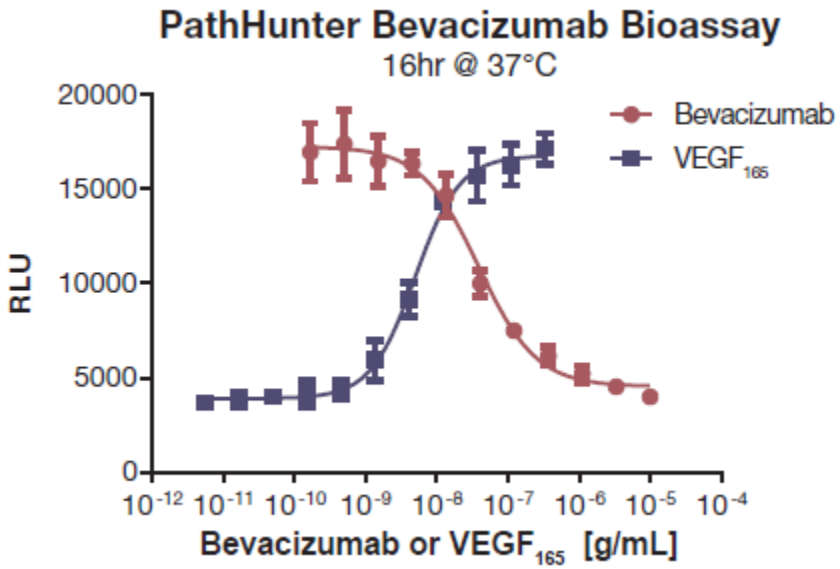
Robust Assay Performance

Better performance than the HUVEC proliferation assay





	HUVEC Proliferation Assay	PathHunter® Bevacizumab Bioassay
EC_{50} VEGF165	8.18 ng/mL	5 ng/mL
IC_{50} VEGF165	67.88 ng/mL	38.96 ng/mL
S:B Ratio	2.5 fold	> 4.3 fold
Assay Run Time	96 hours	16 hours
Cell Type	Primary cells with donor variability	Clonal cryopreserved, ready to use cells

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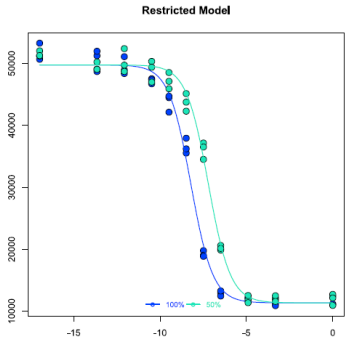
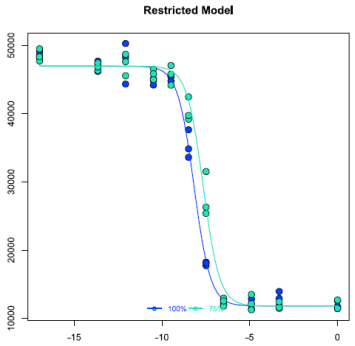
Assay Qualification with Innovator Molecules

Performance of assays for Aflibercept, Bevacizumab, and Ranibizumab

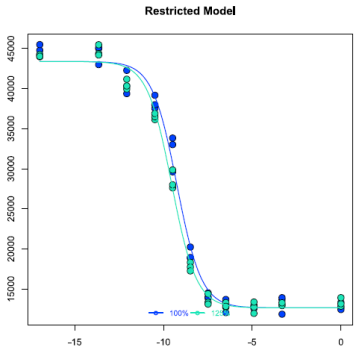
100% vs 50%



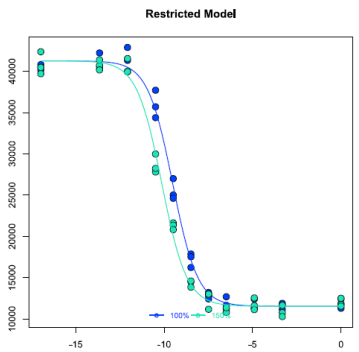
100% vs 75%



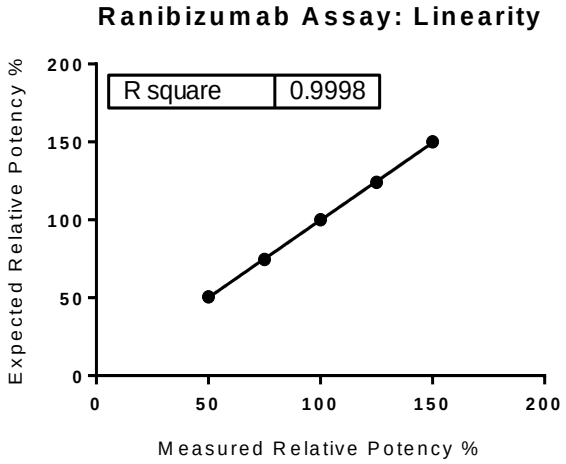
100% vs 125%



100% vs 150%



Relative potency data for ranibizumab



Drug	Accuracy	Precision	Linearity
Aflibercept	96.9%	7.06%	0.976
Bevacizumab	98%	4.8%	0.997
Ranibizumab	96.5%	4.4	0.998

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Cell-Based Assays For Biosimilar Development

DiscoverX has developed assays that respond to 34 biosimilar molecules

DRUG
Ranibizumab
Aflibercept
Bevacizumab
Epoetin Alfa
Darbepoetin Alfa
Exenatide
Liraglutide
Growth Hormone
Filgrastim
Insulin Lispro
Insulin Glargine
Insulin

DRUG
Denosumab
FSH (Follitropin alfa)
LH/hCG
Infliximab
Golimumab
Etanercept
Certolizumab pegol
Adalimumab
GHRH (Somatorelin)
Anakinra
Canakinumab

DRUG
Ustekinumab
Tocilizumab
Glucagon
PTH (Teriparatide)
Secukinumab
Cetuximab
Panitumumab
Pertuzumab
Trastuzumab
Pembrolizumab
Nivolumab

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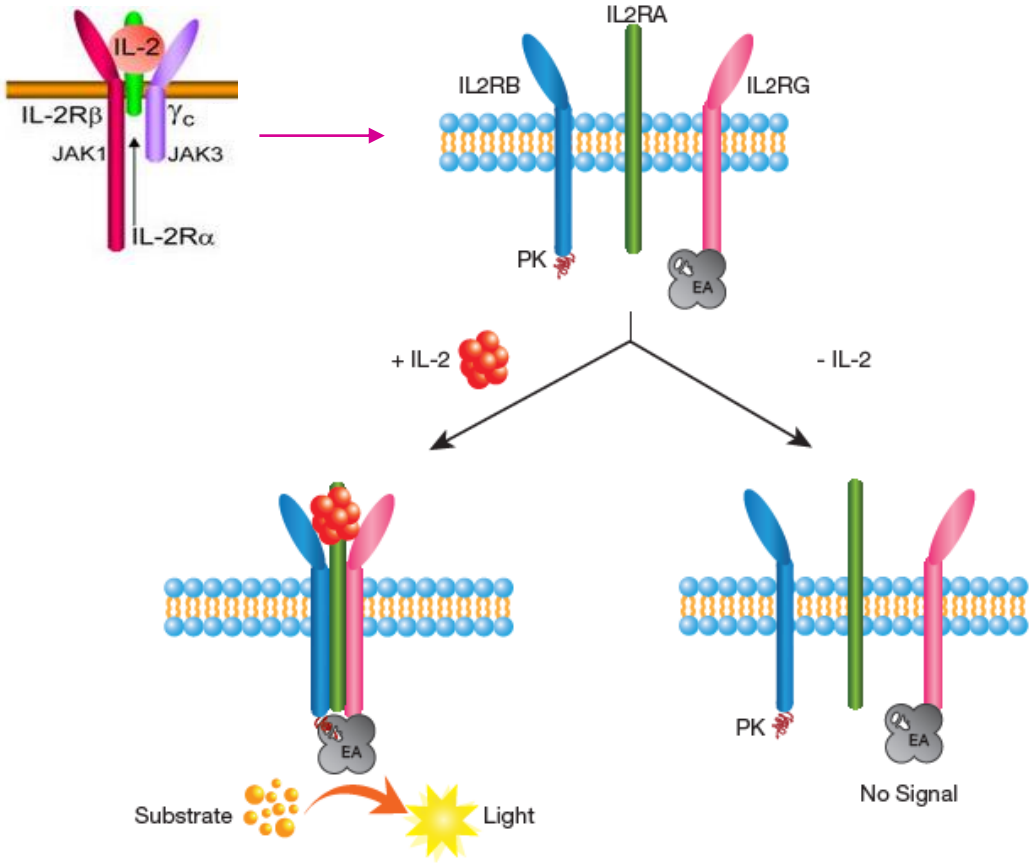
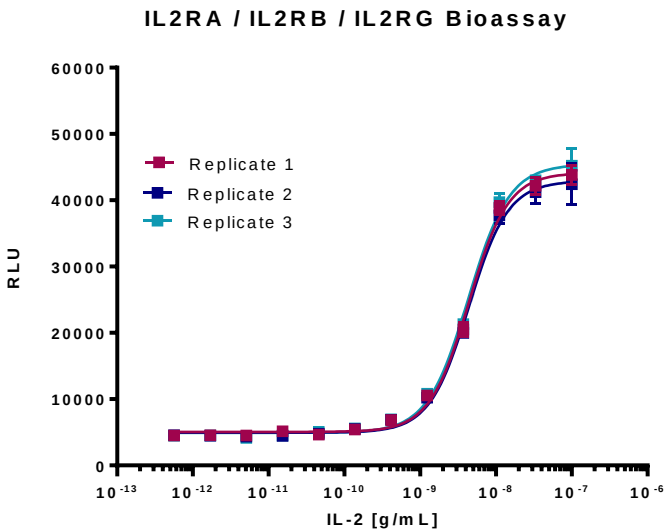
Custom Bioassays for Innovator Molecules

Robust frozen ready-to-use cells for IL-2-based therapeutics





	Replicate 1	Replicate 2	Replicate 3
HillSlope	1.677	1.677	1.603
EC50	4.378e-009	4.374e-009	4.313e-009
S/B	9.6	9.3	10.0

	Analyst 1			Analyst 2		
	Day 1	**Day 2**	**Day 3**	**Day 1**	**Day 2**	**Day 3**
Intra-day	0.87%	8.22%	1.62%	2.40%	8.92%	7.45%
Inter-day			14.33%			6.99%

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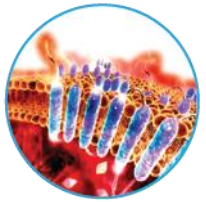
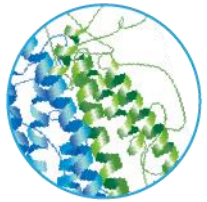
Liao et al. (2011) *Curr Opin Immun* 23(5): 598-604.

Industry's Largest Portfolio of Functional Cell-Based Assays

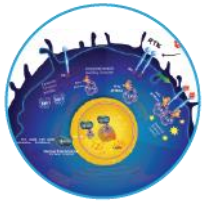
900+ cell lines to support bioassay development for 500+ drug targets



GPCRs



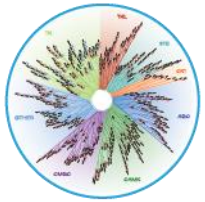
Interleukins



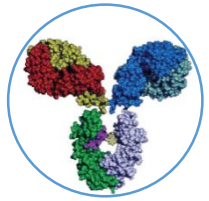
TGFβ Superfamily

Cytotoxicity

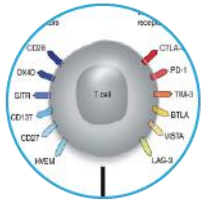
Available Targets	289	55	41	34	14	7	17 + DIY	30+



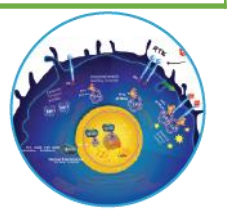
Kinases



Biosimilars



Checkpoint



Others

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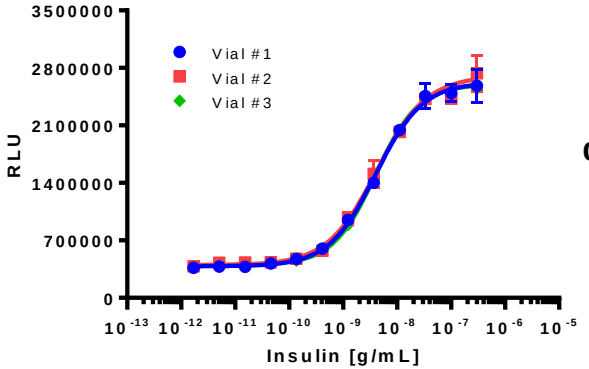
Assays Optimized with Ready-to-Use Cells

Stringent production process for frozen RTU cells to ensure long term results

- ✔ SOP-driven manufacturing process
- ✔ Two-tiered banking of RTU lots
- ✔ Mycoplasma & sterility testing
- ✔ Post-thaw viability
- ✔ Intra-lot functional testing
- ✔ Inter-lot functional testing
- ✔ Documentation provided

Intra-lot precision (Insulin bioassay)



% RSD < 3%

Inter-lot precision (Insulin bioassay)

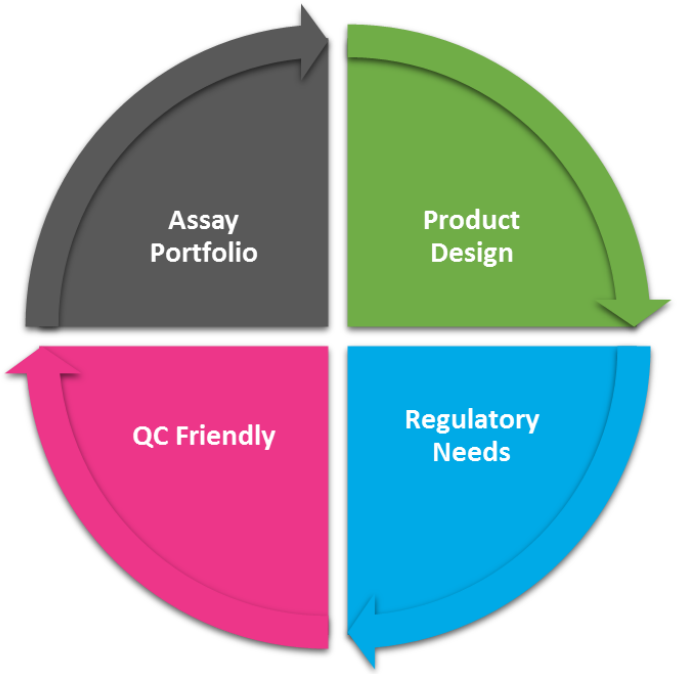
Manufacture date	EC_{50}	Mean EC_{50}	SD	%RSD
2014 - A	1.31×10^{-10}	1.30×10^{-10}	7.6×10^{-12}	5.48
2014 - B	1.46×10^{-10}			
2016	1.14×10^{-10}			

DiscoverX

Benefits of Working with DiscoverX on Bioassays

Over 15 years experience developing cell-based assays

- ✓ End-to-end support for each bioassay project
  - Strong technical support for the life of the project
- ✓ No license limitations from DiscoverX
  - Ability to use assay for commercial release of marketed drug
- ✓ Support transfer globally
  - Ability to ship cell banks directly to sites globally
- ✓ Partnership with multiple CROs globally
  - Hit the ground running with experienced partner CROs
- ✓ Regulatory success with DiscoverX assays
  - Marketed drugs using DiscoverX assays



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Public Partnerships

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SGS

bio outsource
is now part of the
Sartorius Stedim Biotech Group