Ready-to-Use Potency Assays for Bevacizumab, Aflibercept, & Ranibizumab

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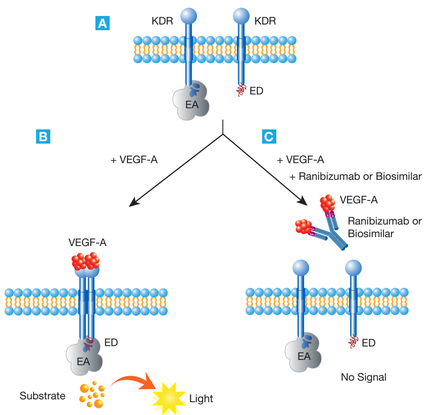
Abstract

Cell-based bioassays often pose a hurdle during a rapidly moving biologics development program. High standards for assay accuracy, precision, reproducibility and robustness are additionally put to the test by the use of continuous culture cells that can add to variability and increase the cost and complexity of each assay. This is particularly challenging for anti-VEGF drugs, as the prevalent assay is the proliferation of primary human umbilical vein endothelial cells (HUVECs), which requires 72-96 hours to run, utilizes primary cells that are difficult to culture and introduces performance variability due to changes in donor, passage number, culture conditions and analyst.

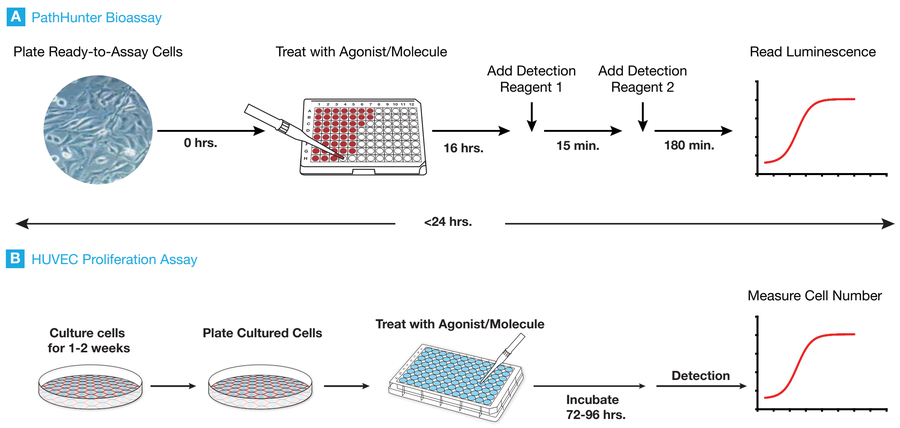
Here, we describe a PathHunter® bioassay that has been developed as a fit-for-purpose potency & stability assay for anti-VEGF drugs. The assay quantifies inhibition of VEGF-A-induced VEGFR2 receptor activation, by measuring an early event in the receptor activation cascade. With its shorter assay time (<24 hours), simple 'add and read' protocol and use of cryopreserved ready-to-assay cells, the PathHunter assay has many advantages over the standard HUVEC assay. Qualification data will be presented on the performance of the PathHunter bioassay for bevacizumab, aflibercept and ranibizumab.

Mechanism of Action-Based Bioassay Design

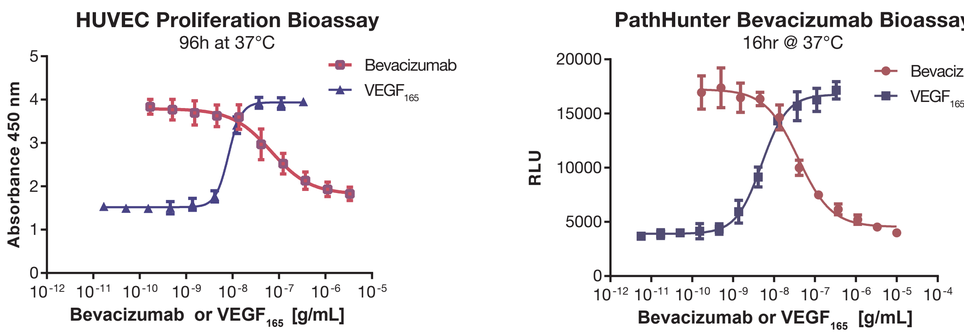


A. The PathHunter bioassay for VEGFR2 targets an early event in VEGFR2 signaling, measuring VEGF-A–induced homodimerization of the VEGFR2 receptor. The assay utilizes DiscoverX' proprietary Enzyme Fragment Technology consisting of two fragments of β-galactosidase (β-gal) – ED & EA – which are inactive when apart. Two VEGFR2 receptors are tagged with the inactive fragments and stably engineered into a human cell line. B. Upon activation, by VEGF-A the VEGFR2 receptors naturally dimerize forcing the two β-gal fragments to complement and create an active enzyme. Active β-gal hydrolyzes its substrate and produces a chemiluminescent signal, indicating receptor activation C. Anti-VEGF molecules like aflibercept, bevacizumab or ranibizumab inhibit VEGF-A's ability to activate the receptors and therefore inhibit the chemiluminescent signal

Complete Entire Assay in Less Than 24 Hours

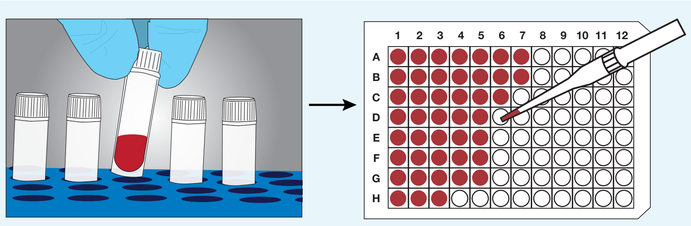


HUVEC Proliferation Assay vs. PathHunter Bevacizumab Bioassay



	HUVEC Proliferation Assay	PathHunter Bevacizumab Bioassay
EC_{50} VEGF165	8.18 ng/mL	6.3 ng/mL
EC_{50} Bevacizumab	67.88 ng/mL	76.7 ng/mL
S:B Ratio	2.5 fold	4-4.5 fold
Assay run time	96 hours	16 hours
Cell type	Primary cells with donor variability	Clonal, frozen ready-to-assay cells
Cell Culture	Required	No cell culture necessary

Use of Cryopreserved Ready-to-Use Cells

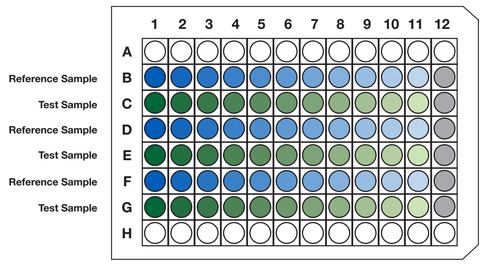


Frozen Ready-to-Use cells

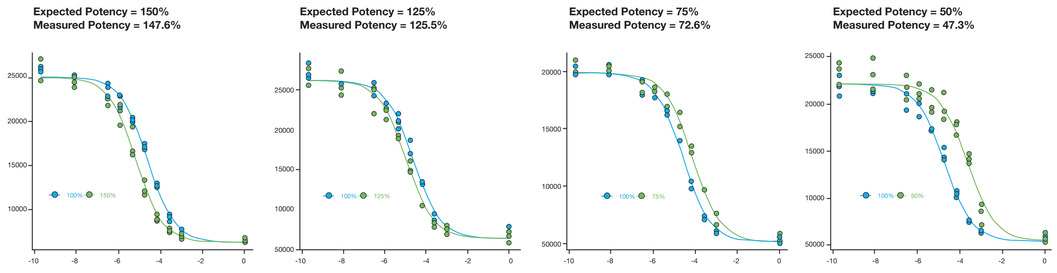
- Higher efficiency
- Greater assay reproducibility
- Operational flexibility
- Cost savings - Serum, culture media and reagents
- Ease of assay development and method transfer to global sites

PathHunter 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 personnel are needed for cell maintenance and media preparation, more consistent assay performance as all the cells in a 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.

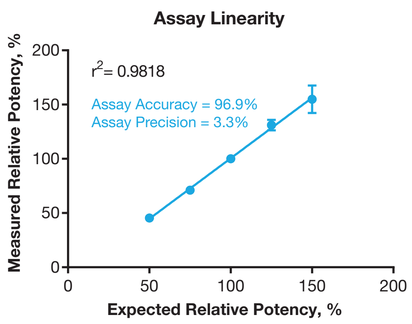
Bioassay Qualification with Aflibercept



Left: Plate design of the bioassay.
Bottom middle: Dose response curves in PLA software showing expected and measured potencies for 4 samples, ranging from 50% to 150% potencies.
Bottom: Table of qualification data with Aflibercept, measuring test samples ranging from 50% to 150% run on three different days by one analyst.

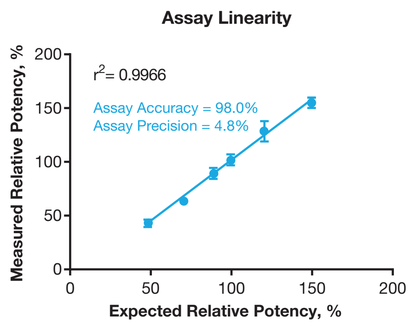


Day	Expected Potency (%)	Measured Potency (%)	Mean Potency (%)	Recovery (%)	RSD (%)
1	150	147.6	154.9	103.3	12.7
2		169.6			
3		147.6			
1	125	125.5	131.1	104.9	4.9
2		134.8			
3		132.9			
1	75	72.4	71.1	94.8	2.4
2		72.6			
3		68.3			
1	50	47.3	45.5	91	2.6
2		42.5			
3		46.6			



Bioassay Qualification with Bevacizumab

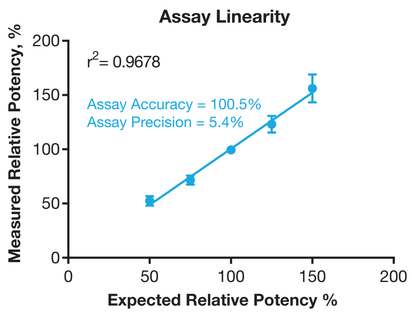
Day	Expected Potency (%)	Measured Potency (%)	Mean Potency (%)	Recovery (%)	RSD (%)
1	150	161.5	155.5	103.7	2.3
2		155.4			
3		152.6			
4		152.6			
1	121	132.5	128.3	106.0	7.1
2		136.3			
3		112.9			
4		131.3			
1	100	107.1	102.1	102.1	5.3
2		106.2			
3		93.4			
4		101.8			
1	90	83.3	89.8	99.7	5.3
2		88			
3		91.4			
4		96.3			
1	71	64.3	63.9	90.0	1.1
2		62.9			
3		64.8			
4		63.6			
1	50	48.7	43.3	86.7	7.4
2		42.4			
3		41.9			
4		40.3			



Here we have tested the PathHunter Bevacizumab bioassay with Avastin® as a reference molecule, demonstrating a robust response and a high level of reproducibility with multiple runs.The VEGFR dimerization assay was tested with six test samples, from 50% to 150%, compared to a reference standard (100%) by one operator over a course of four days. The measured relative potencies were plotted against the expected relative potencies with a very high degree of linearity, accuracy and precision.

Bioassay Qualification with Ranibizumab

Day	Expected Potency (%)	Measured Potency (%)	Mean Potency (%)	Recovery (%)	RSD (%)
1	150	172.1	157.2	104.8	13.4
2		146.2			
3		153.2			
1	125	132.2	123.8	99	7.7
2		117.2			
3		121.9			
1	75	74.1	72.3	96.4	4.2
2		75.3			
3		67.5			
1	50	57.9	53.0	106	4.4
2		51.7			
3		49.5			



Summary

- Functional assay for anti-VEGF drugs based on their mechanism of action
- Simple, homogenous protocol with results in less than 24 hours
- Excellent potency and linearity with high accuracy and precision
- Highly reproducible and robust with cryopreserved ready-to-assay cells
- Significantly better assay performance compared to difficult HUVEC assay
- Suitable for comparability studies, lot release testing, stability studies and drug characterization

Go to discoverx.com/biosimilars to see additional assays and verification data

