Ready-to-use potency assays for anti-VEGF drugs such as Bevacizumab

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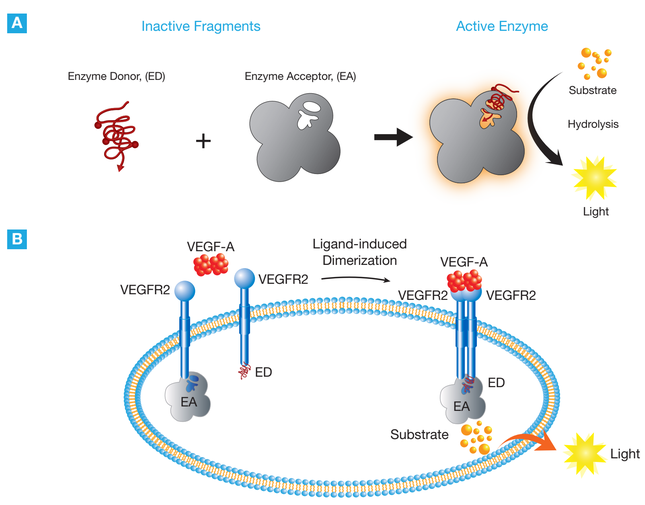
Abstract

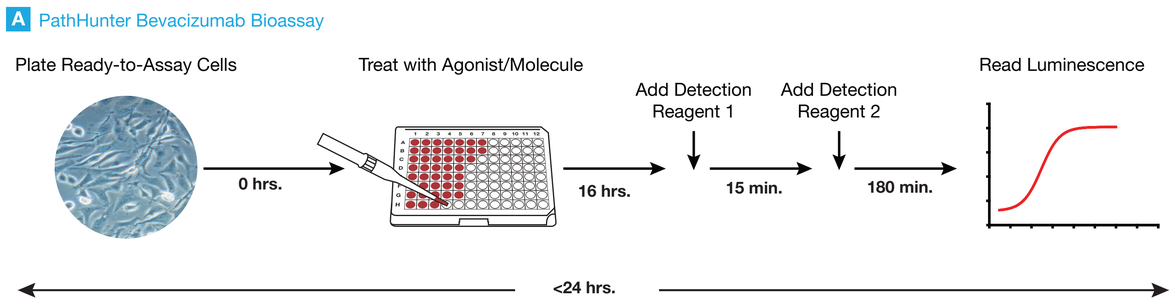
Cell-based bioassays often pose a hurdle during a rapidly moving biologics development program. High standards for assay accuracy, precision, reproducibility and robustness are additionally put to the test by the use of continuous culture cells that can add to variability and increase the cost and complexity of each assay. This is particularly challenging for anti-VEGF drugs, as the prevalent assay is the proliferation of human umbilical vein endothelial cells (HUVECs), which requires 72-96 hours to run, utilizes cells that are difficult to culture and introduces performance variability due to changes in passage number, culture conditions and operator. Here, we describe the PathHunter® bioassay that has been developed as a fit-for-purpose QC Lot Release assay for anti-VEGF drugs. The assay quantifies inhibition of VEGF-A-induced VEGFR2 receptor activation, by measuring receptor dimerization as 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. Data will be presented comparing the performance and reproducibility of the PathHunter Bevacizumab bioassay to the standard HUVEC proliferation assay.

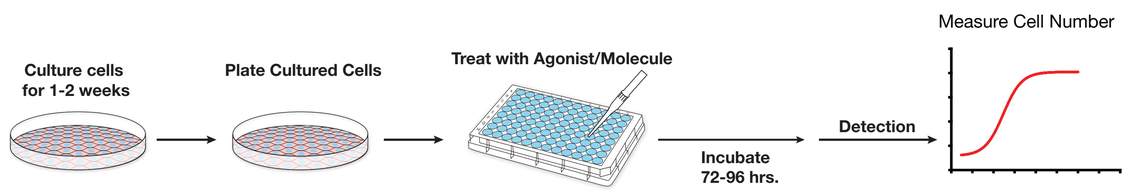
Introduction to PathHunter Bevacizumab Bioassay



A. 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. B. Two VEGFR2 receptors are tagged with ED & EA, creating an engineered cell line that will respond to receptor homodimerization.Upon activation, by VEGF-A the VEGFR2 receptors naturally dimerize forcing the two β-gal components (ED & EA) to complement and create an active enzyme. Active β-gal generates a chemiluminescent signal in the presence of substrate, signaling the activation of the VEGFR2 receptors.

Assay Protocol



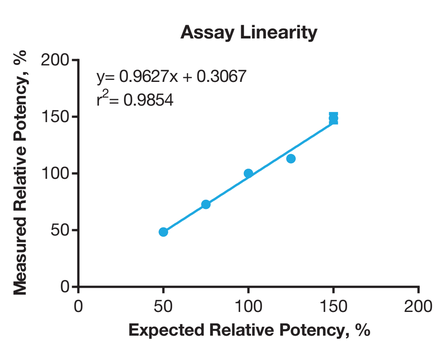


PathHunter Bioassay Reproducibility, Accuracy, Precision & Linearity

Assay Accuracy & Precision

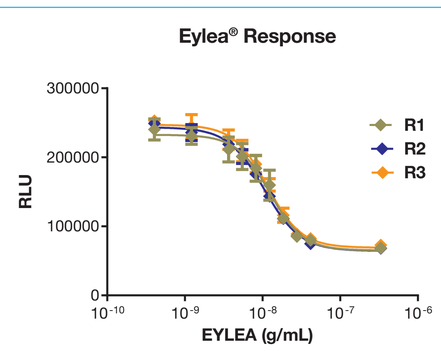
	Expected Potency, %	Measured Potency, %	Mean Potency, %	SD	Recovery, %	RSD, %
Day 1	150	151.0	148.7	4.04	99.1	5.12
Day 2		144.0				
Day 3		151.0				
Day 1	125	109.0	113.0	3.46	90.4	3.07
Day 2		115.0				
Day 3		115.0				
Day 1	75	74.4	72.7	2.47	97.0	3.12
Day 2		73.9				
Day 3		69.9				
Day 1	50	48.8	48.5	2.42	96.9	4.99
Day 2		45.9				
Day 3		50.7				

Accuracy= 95.9% Precision= 4.1%

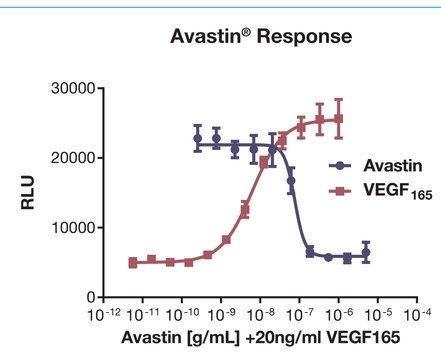


Here we have tested the PathHunter Bevacizumab bioassay with VEGF-A, demonstrating a robust response and a high level of reproducibility with multiple runs.The VEGFR dimerization assay was tested with four test samples, from 50% to 150%, compared to a reference standard (100%) by one operator over a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

Potency of Therapeutic Molecules with PathHunter Bioassay

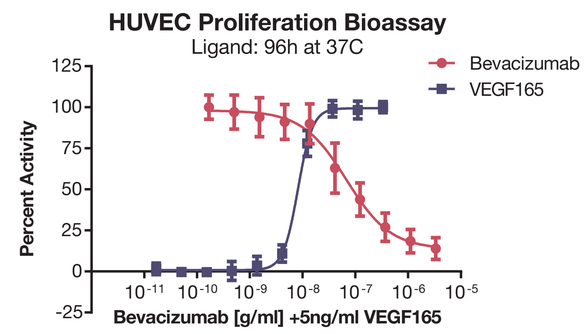


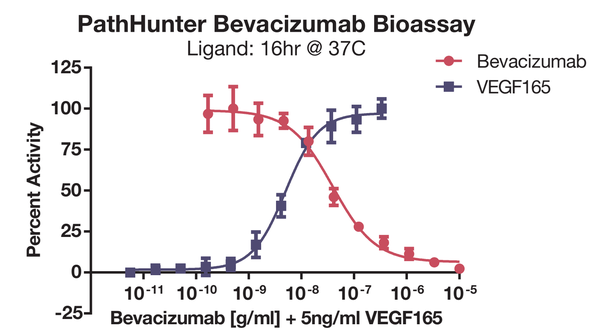
	Repeat 1	Repeat 2	Repeat 3
EC_{50}(ng/ml)	12.4	10.5	11.5
S/B Ratio	3.6	3.8	3.6



	Avastin	VEGF165
EC_{50}	7.67e-008	6.33e-009
S/B	4.0	5.1

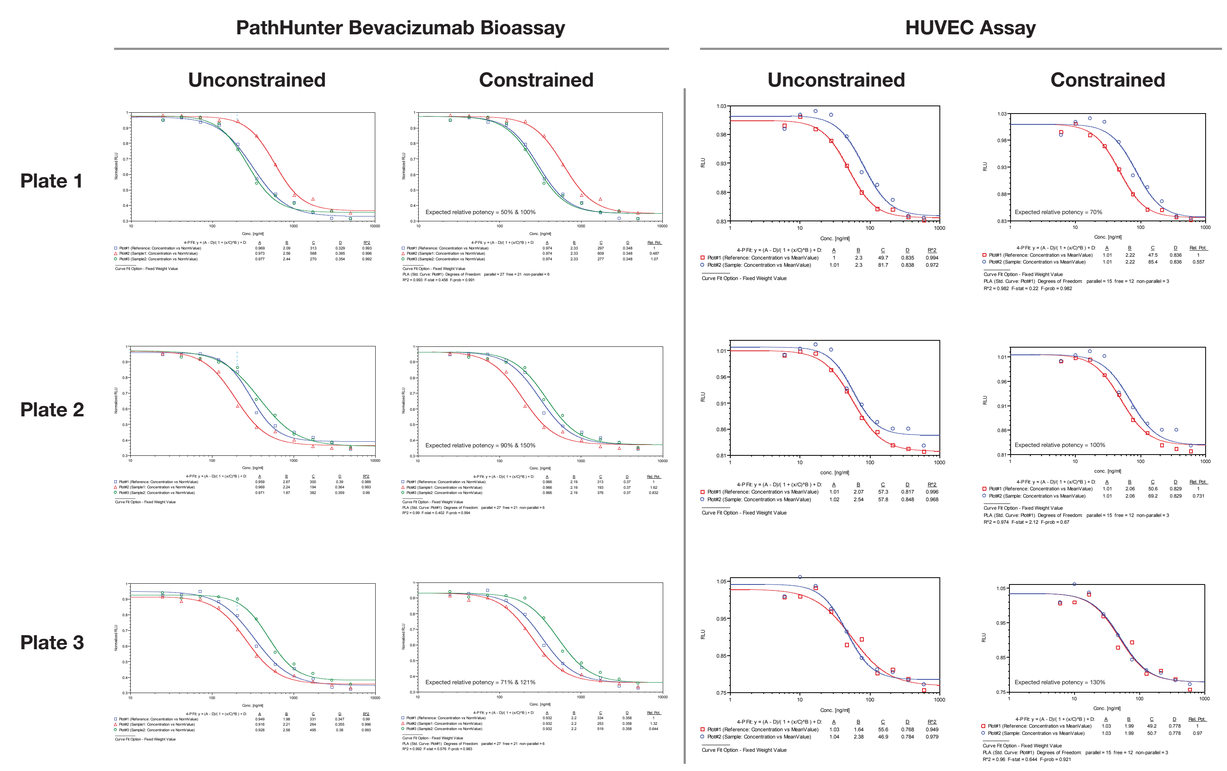
HUVEC Proliferation Assay vs. PathHunter Bevacizumab Bioassay





	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
Specificity	Low	High
Assay Output	MTT Readout	Chemiluminescence
Cell type	Primary cells with donor variability	Clonal, frozen ready-to-assay cells
Cell Culture	Required	No cell culture necessary

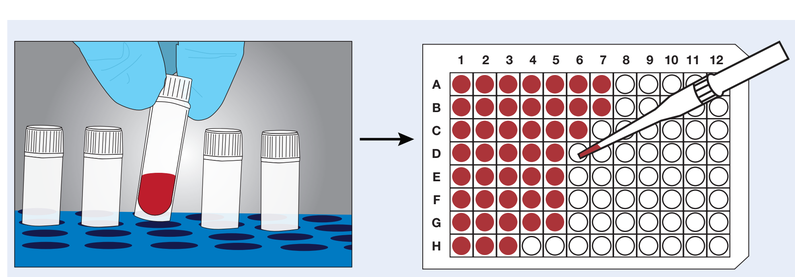
Here we have tested the PathHunter Bevacizumab bioassay with VEGF-A and bevacizumab (top right), demonstrating the robust and precise response to both agents, and EC/IC-50's comparable to those obtained in the HUVEC proliferation assay (top left). The table below compares the proliferation assay and the PathHunter assay, demonstrating that the latter is quicker, more robust, highly specific and generates the better data without the need for any cell culture.



Expected Potency %	Assay Potency %
50	48.7
71	64.3
90	83.3
100	107.1
121	132.5
150	161.5

Expected Potency %	Assay Potency %
70%	55.7%
100%	73.1%
130%	97.0%

Use of Cryopreserved Ready-To-Assay 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-assay 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.

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 lot release & stability studies and drug characterization

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

