eurofins | DiscoverX

Accelerating Biosimilar & Biobetter Drug Development

Ready-to-Use Cell-Based Assays for Comparability and QC Lot Release Testing

Ready-to-Use Cell-Based Assays for Biosimilars

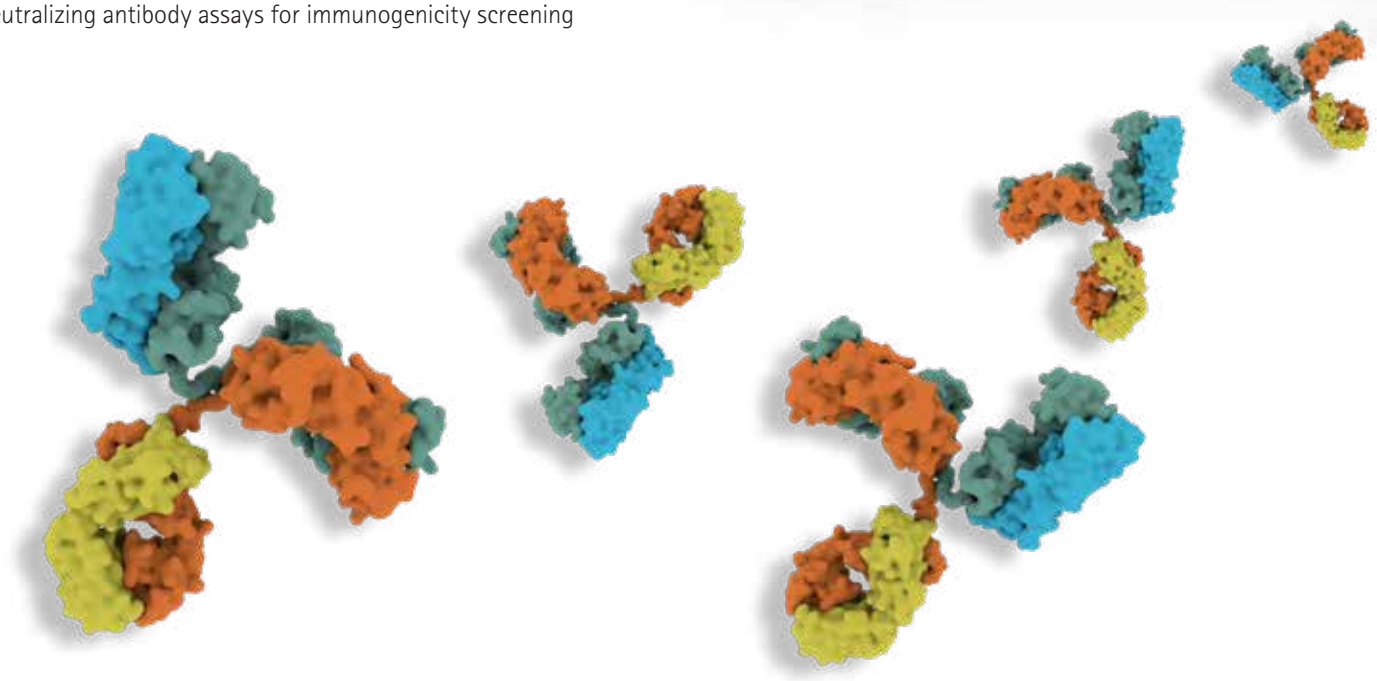
A new era of biologic drugs has begun. Biosimilars and biobetters are approved follow-on versions of innovator biologics that provide opportunities for reducing the cost of treatment without compromising quality.

The development pathway of a biosimilar or a biobetter is different from that of novel biotherapeutics, requiring increased bioanalytical testing. Requirements for comparability, efficacy, and potency testing often demand a complex set of bioassays and/or cell-based assays for biosimilars and biobetters. Developers of these follow-on biologics have found this need for cell-based assays to be particularly challenging.

Eurofins DiscoverX addresses this challenge with commercially available, ready-to-use cell-based assays for >30 biosimilar targets. These qualified fit-for-purpose, off-the-shelf assays save drug developers at least 6 months of assay development time, accelerating a path to regulatory submission. Initiate and complete comparability and method development with an easy-to-use assay protocol that generates rapid results in 24-48 hours, increasing overall assay reproducibility and ease of transfer, globally.

Applications of Eurofins DiscoverX Bioassays Include

- in vitro comparability testing
- Characterization studies
- Potency assay development
- Quality control testing for lot release
- Drug stability testing
- Neutralizing antibody assays for immunogenicity screening



Simplified Comparability and QC Lot Release Testing

Benefits of Eurofins DiscoverX Bioassays

- Implement biologically-relevant, mechanism of action (MOA)-based assays for over 30 biosimilars
- Drive rapid implementation of qualified fit-for-purpose assays, available as cell lines or frozen ready-to-use cells
- Experience effortless method development & transfer with easy-to-use protocol
- Increase lab efficiency and minimize cycle time with results in 24-48 hours
- Support long-term bioassays through two-tiered cell banks and included license for commercial drug release

Eurofins DiscoverX Bioassays Are Globally Adopted by the Pharma & Contract Service Industry

- Used for the lot release of a marketed drug in APAC and EMA
- Submitted in IND filings by multiple pharmaceutical companies
- In the development pipeline for lot-release testing of many biosimilar and innovator molecules, globally
- Covance, Catalent, SGS, Vela Labs, & BioOutsource are among many service providers offering services with Eurofins DiscoverX bioassays

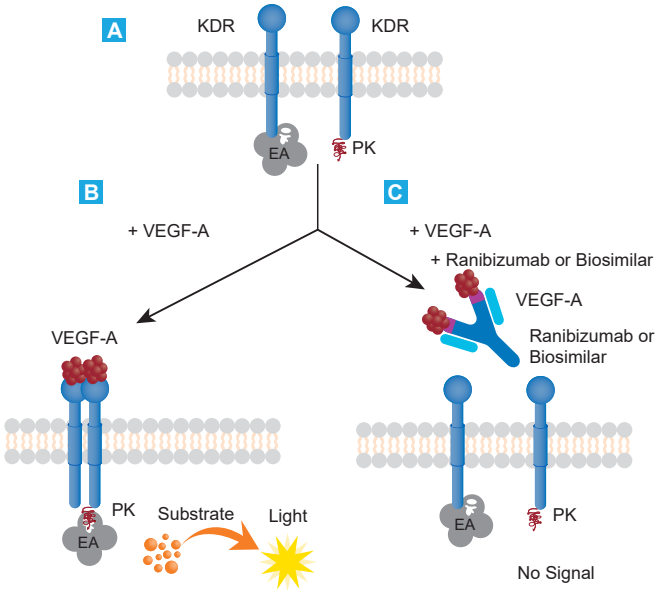
Case Study 1

Bioassay for Aflibercept & Other Anti-VEGF Molecules

Bevacizumab, aflibercept, and ranibizumab together account for billions of dollars of drug sales. The most common assay for the lot release of these molecules is the proliferation of HUVEC cells, which takes 4 days to run and requires the use of primary cells. These factors lead to poor precision and high variability due to donor heterogeneity, cell culture method, and the general challenges of working with primary cells.

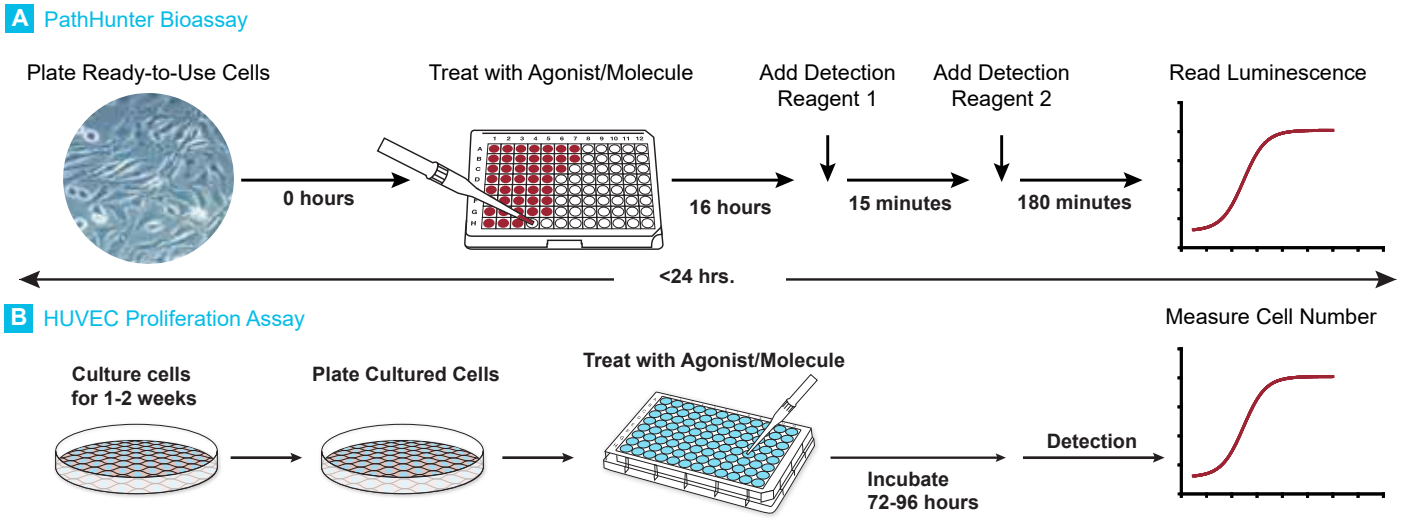
The PathHunter® bioassays for bevacizumab, aflibercept, and ranibizumab present robust alternatives, with MOA-based bioassays developed from a stable cell line, delivering rapid and highly reproducible results in less than 24 hours.

Mechanism of Action-Based Assay for Aflibercept



The PathHunter Aflibercept Bioassay targets an early event in KDR signaling, measuring VEGF-A–induced homodimerization of the KDR receptor. The assay utilizes Eurofins DiscoverX's proprietary Enzyme Fragment Technology consisting of two fragments of β-galactosidase (β-gal) – PK & EA – which are inactive when apart. Two KDR receptors are tagged with the inactive fragments and stably engineered into a human cell line. Upon activation, by VEGF-A the KDR 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. 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



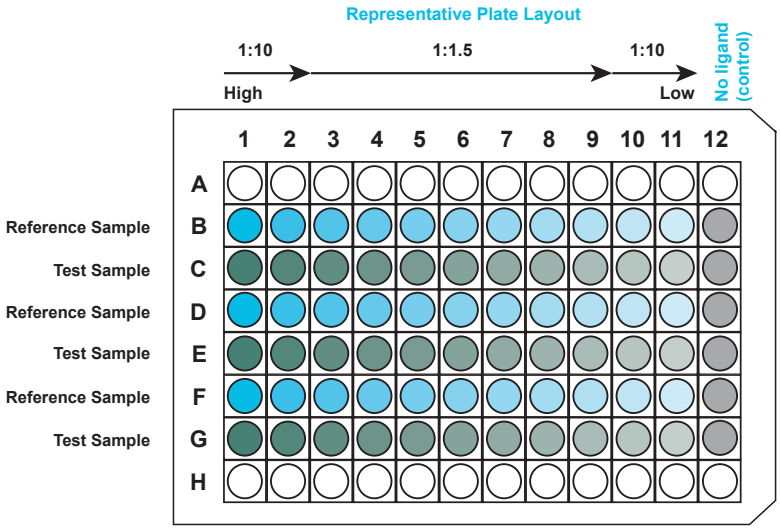
Protocol comparison. A. The PathHunter Aflibercept Bioassay uses cryopreserved ready-to-use cells that do not require any cell culture. Using this approach, the entire assay can be completed in less than 24 hours with a simple add-and-read protocol. B. In comparison, the HUVEC proliferation assay takes 1-2 weeks to culture cells prior to using these cells in the proliferation assay that takes 3-4 days to run. Reduce assay time from 2-3 weeks to less than 1 day with the PathHunter bioassays.

Case Study 1 *continued...*

Qualification of PathHunter® Aflibercept Bioassay

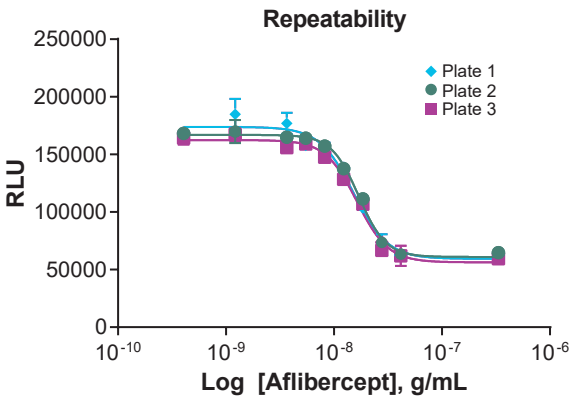
Design

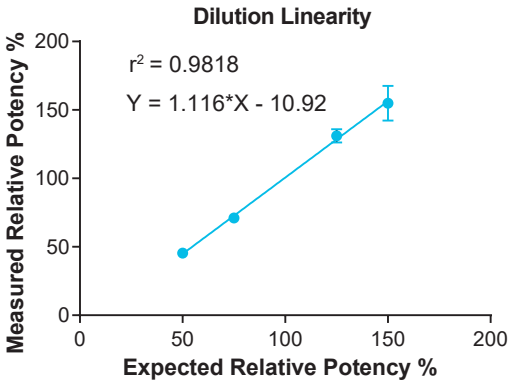
- Two analysts
- Three days
- Four plates per day
- Reference (100%) vs 50%
- Reference (100%) vs 75%
- Reference (100%) vs 125%
- Reference (100%) vs 150%



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

Precision = average of RSD (%) = 3.3% Accuracy = average of Recovery (%) = 96.9

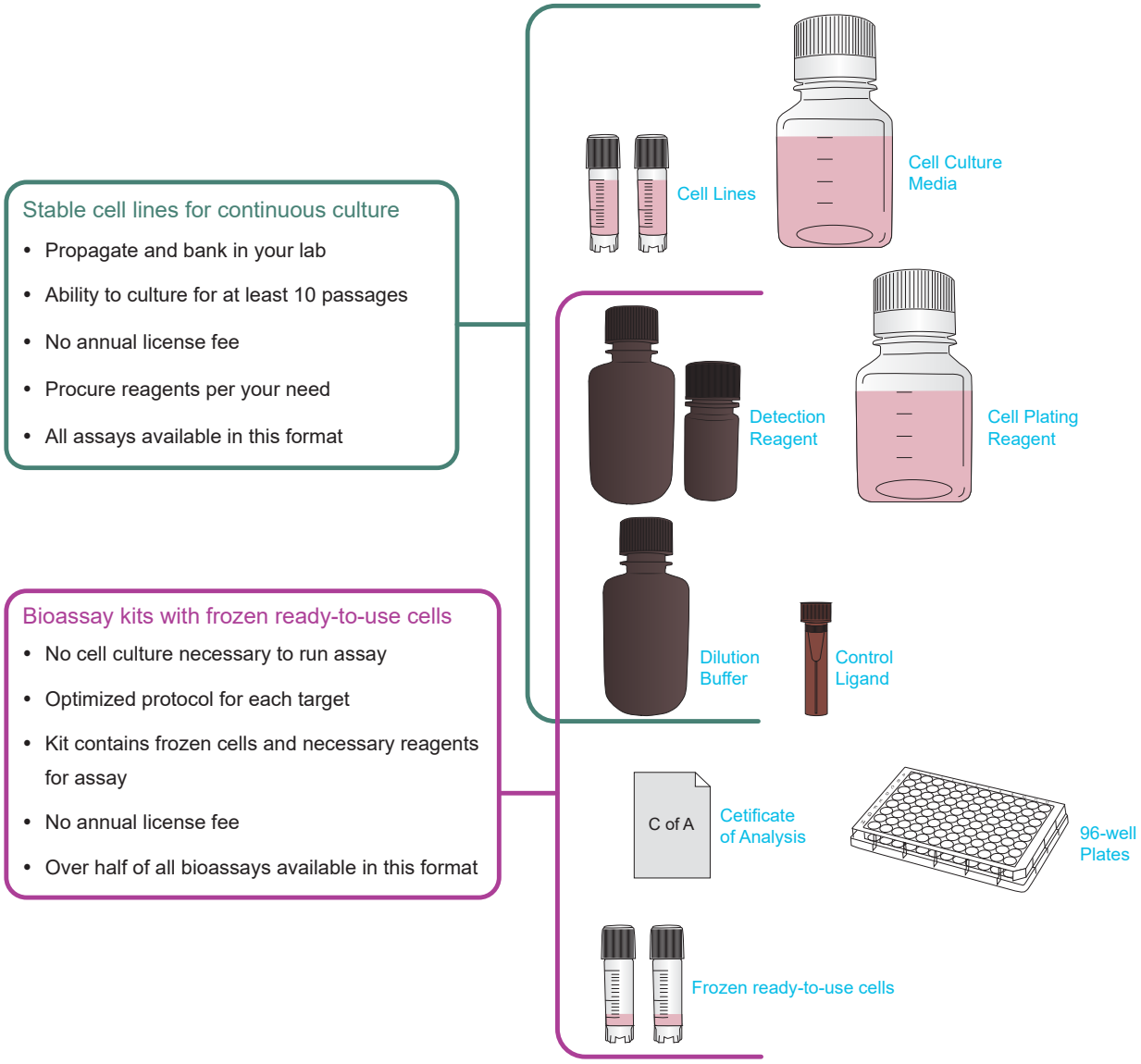




To assess the suitability of the PathHunter Aflibercept Bioassay for potency testing, we performed an assay qualification exercise over three days with frozen ready-to-use cells. The above assay design was used with the representative plate layout as shown above. The assay demonstrated an excellent inter-plate repeatability with 4.9% CV for IC_{50} values. The PathHunter Aflibercept Bioassay had superior accuracy and precision, with good dilutional linearity.

Eurofins DiscoverX Bioassay Product Formats

Flexible Formats to Fit the Needs of Your Organization



Advantages of Frozen Ready-to-Use Cells in Bioassay Kits

- Increased efficiency - fewer personnel needed for cell maintenance and media preparation
- Data consistency and assay reproducibility - all cells in a given bank are frozen at the same passage number
- Cost savings - reduce personnel time, culture media and reagents
- Ease of assay development and method transfer to global sites - handling of cells during continuous culture can account for many transfer problems
- Operational flexibility - assays can be performed as and when needed

For ordering information for any cell line or bioassay kit, visit discoverx.com/bioassays

Available Bioassays for Biosimilar & Biobetter Drugs

Save More Than 6 Months of Assay Development Time with these Ready to Use Bioassays

Inn Drug Name	Qualified With	Part Number
		(2-Plate Kit & 10-Plate Kit)*
Bevacizumab	Avastin®	93-0996Y1-00001 & -00002
Ranibizumab	Lucentis®	93-0996Y1-00003 & -00004
Aflibercept	Eylea®	93-0996Y1-00005 & -00006
Anakinra	Kineret®	93-1032Y3-00105 & -00106
Darbepoetin Alfa	Aranesp®	93-0965Y3-00019 & -00020
Epoetin Alfa	Recombinant hEpo	93-0965Y3-00017 & -00018
Exenatide	Byetta®	95-0062Y2-00101 & -00102
Liraglutide	Victoza®	95-0062Y2-00099 & -00100
Follitropin alfa	Gonal-F	95-0119Y2-00103 & -00104
Insulin	USP Insulin	93-0466Y3-00007 & -00008
Insulin Glargine	USP Insulin	93-0466Y3-00011 & -00012
Insulin Lispro	USP Insulin	93-0466Y3-00009 & -00010
Panitumumab	Vectibix®	93-1051Y3-00093 & -00094
Parathyroid Hormone (PTH)	Recombinant hPTH	93-0315Y2-00047 & -00048
Anti-PD-1 & PD-L1 drugs	Keytruda®	93-1104Y19-00117 & -00118
Pertuzumab	Perjeta®	93-1042Y3-00095 & -00096
Sargramostim	Leukine®	93-1078Y3-00111 & -00002
Somatotropin, Somatropin	Recombinant hGH	93-0756Y3-00023 & -00024
Teriparatide	Forteo®	95-0118Y2-00057 & -00058

For pricing information, ordering and an up-to-date assay list, visit discoverx.com/bioassays

w discoverx.com usa 1.866.448.4864 eu +44.121.260.6142 e CustomerServiceDRX@eurofins.com