

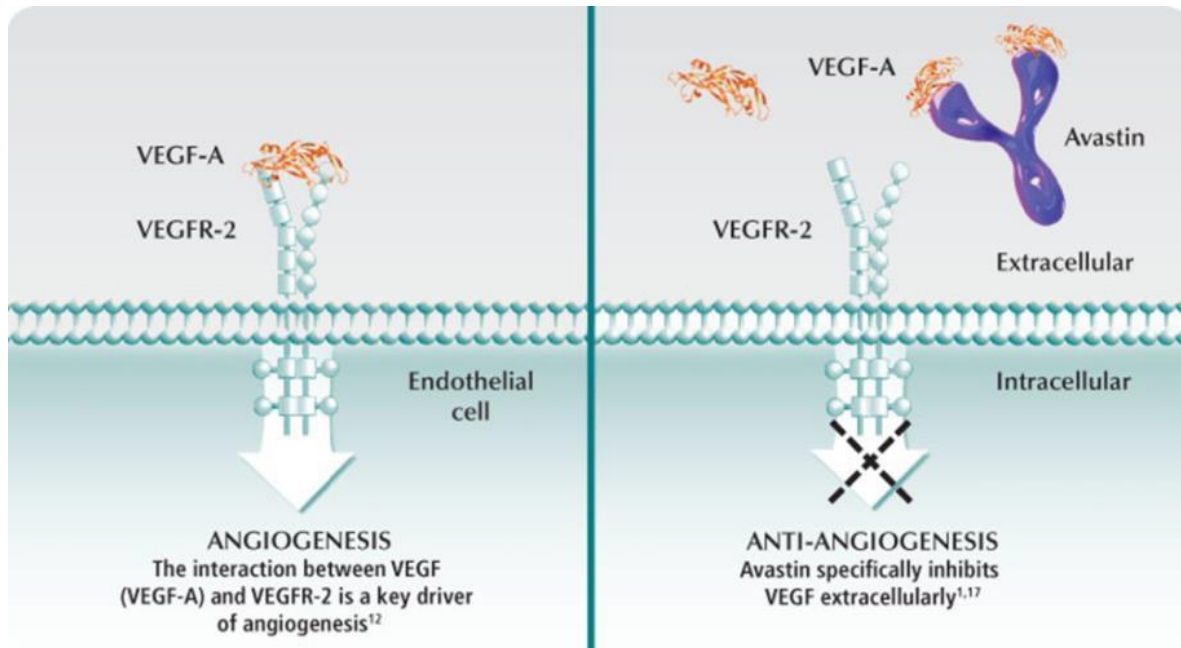


# Qualified Bioassay Kits for anti-VEGF Biologics

Bevacizumab , Ranibizumab, Aflibercept

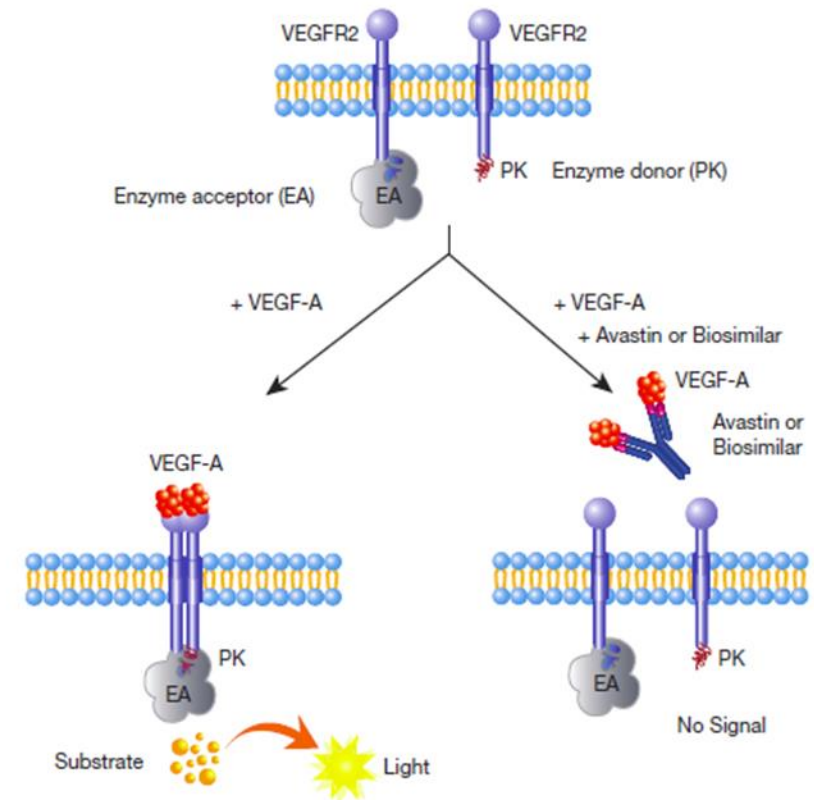
OUR EXPERTISE  
IN YOUR HANDS.  
DISCOVER  
CONFIDENTLY.

## Mechanism of Action e.g. Bevacizumab (Avastin<sup>®</sup>)



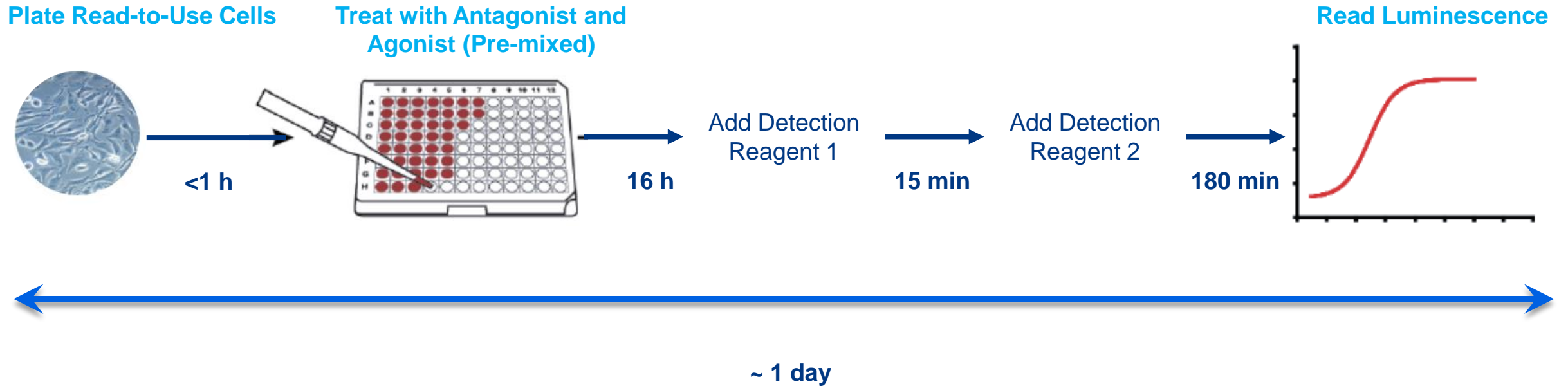
(Image Source: [www.avastin-hcp.com](http://www.avastin-hcp.com))

## Assay Principle



# Bioassay Workflow

*Simple, Homogenous and Rapid Protocol*



## *Assay Parameters Assessed*

- % CV between 8 full plate DRCs
- Plate uniformity:  $EC_{80}$  and  $IC_{80}$  (of drug and stimulus) across entire plate
- Plate-to-Plate variability: 3 plates with full plate DRCs run on 3 days
- Slope consistency
- Relative potency across range of 50-150%
- Parallelism of relative potency data from two operators
  - Assay developer
  - Assay qualifier

## Bevacizumab Bioassay

[93-0996Y1-00001](#) (2-Plate Kit)

[93-0996Y1-00002](#) (10-Plate Kit)

Qualified with Avastin<sup>®</sup>



[Bevacizumab Bioassay Qualification data](#)

## Ranibizumab Bioassay

[93-0996Y1-00003](#) (2-Plate Kit)

[93-0996Y1-00004](#) (10-Plate Kit)

Qualified with Lucentis<sup>®</sup>



[Ranibizumab Bioassay Qualification data](#)

## Aflibercept Bioassay

[93-0996Y1-00005](#) (2-Plate Kit)

[93-0996Y1-00006](#) (10-Plate Kit)

Qualified with Eylea<sup>®</sup>



[Aflibercept Bioassay Qualification data](#)

Avastin<sup>®</sup> and Lucentis<sup>®</sup> are registered trademarks of Genentech. Eylea<sup>®</sup> is a registered trademark of Regeneron Pharmaceuticals, Inc.

Sample dataset

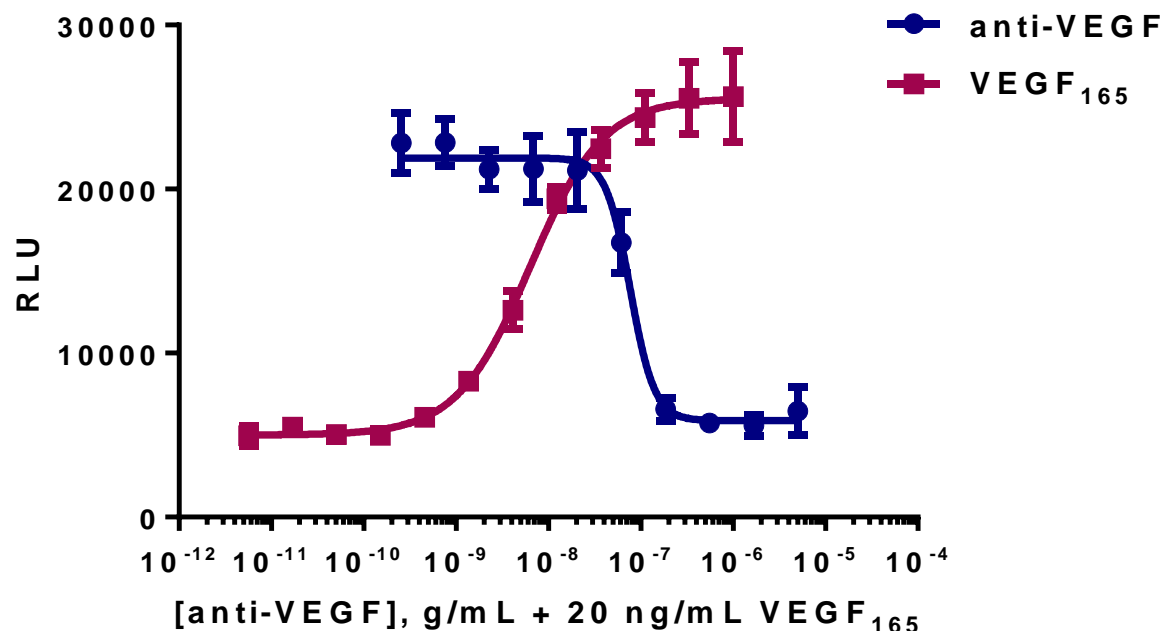
# Bevacizumab Bioassay Qualification

# PathHunter<sup>®</sup> Bevacizumab Bioassay Kit

## Kit Components

List of Components	93-0996Y1-00001	93-0996Y1-00002
PathHunter HEK 293 KDR/KDR Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit	200 dp	1,000 dp
Detection Reagent 1	2 mL	10 mL
Detection Reagent 2	8 mL	40 mL
AssayComplete Cell Plating Reagent 0	1 X 100 mL	3 X 100 mL
Protein Dilution Buffer	1 X 50 mL	2 X 50 mL
Control Agonist (VEGF <sub>165</sub> )	1 vial	1 vial
96-Well Opaque-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

# Typical response to agonist and anti-VEGF molecules

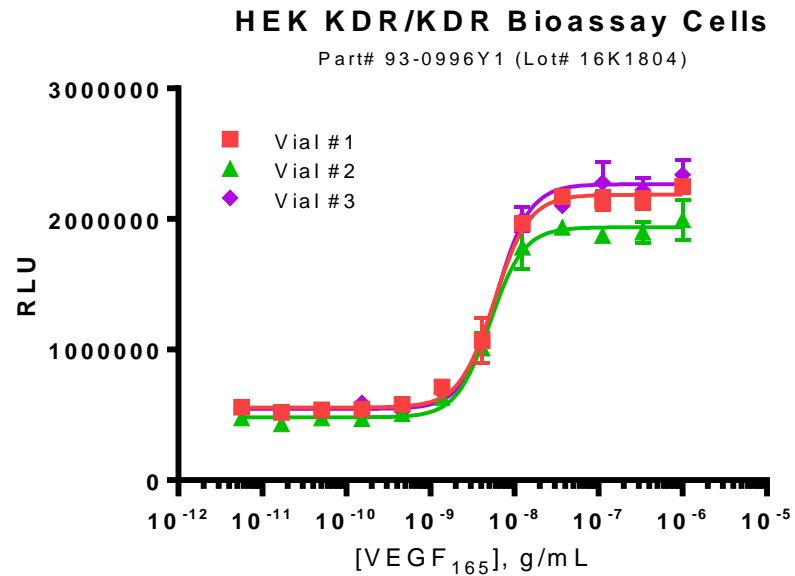


	Bevacizumab	VEGF <sub>165</sub>
Hill Slope	-3.285	1.098
EC <sub>50</sub> (ng/mL)	76.75	6.337
S/B	4.0	5.1



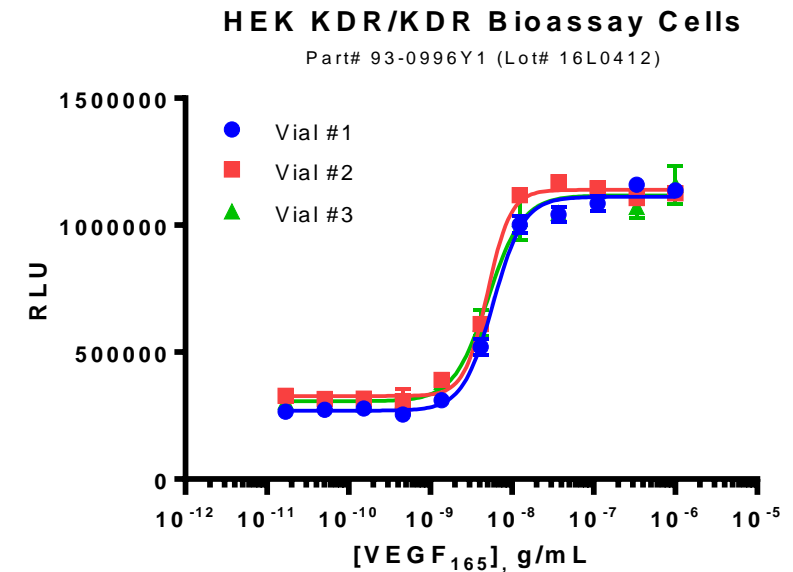
# Robust Bioassay Manufacturing Ensures Consistent Performance

## Inter-Lot and Intra-Lot Reproducibility



	Vial 1	Vial 2	Vial 3
<b>Hill Slope</b>	2.087	2.173	2.166
<b>EC<sub>50</sub> (ng/mL)</b>	5.72	5.1	6.01
<b>S/B</b>	4.0	4.2	4.3

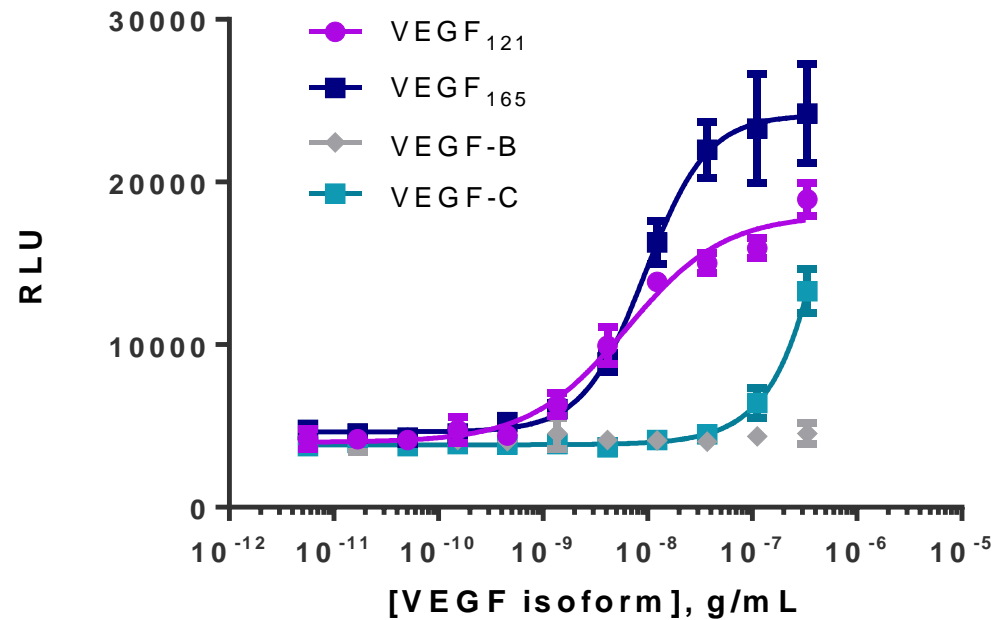
% RSD (EC<sub>50</sub>) = 8.31%



	Vial 1	Vial 2	Vial 3
<b>Hill Slope</b>	2.327	3.051	2.057
<b>EC<sub>50</sub> (ng/mL)</b>	5.84	4.98	5.07
<b>S/B</b>	4.4	3.8	3.9

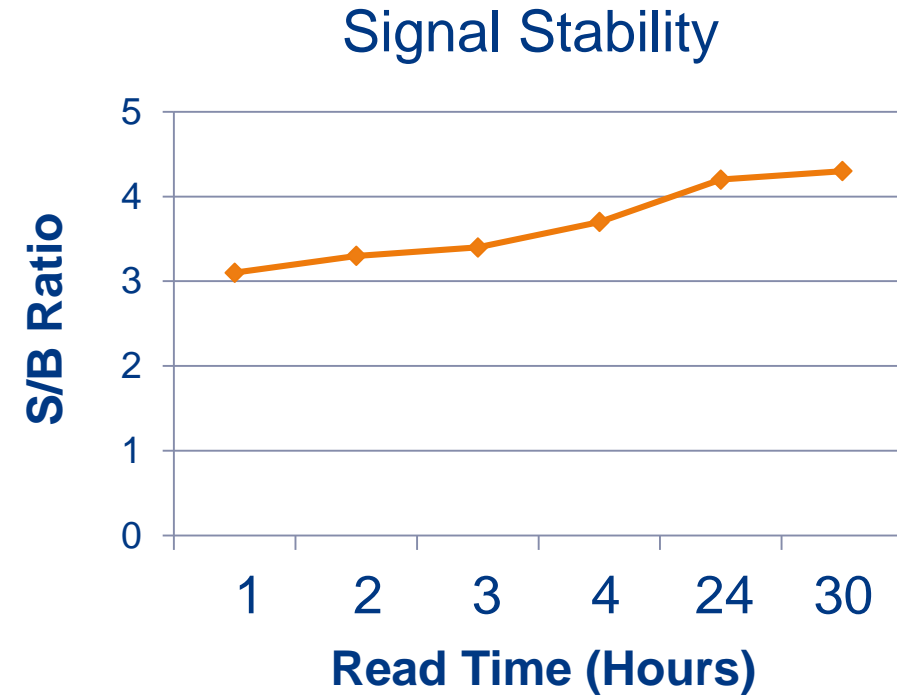
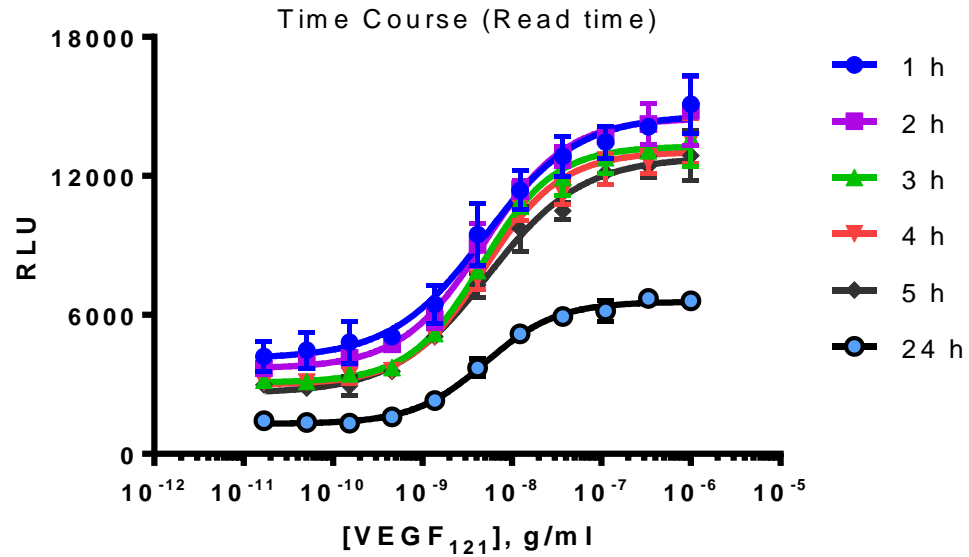
% RSD (EC<sub>50</sub>) = 9.02%

# Bioassay is Specific for VEGF-A isoforms and VEGF-C



- The assay is specific for VEGF-A isoforms (VEGF<sub>121</sub> and VEGF<sub>165</sub>)
- Some weak activation is observed from VEGF-C
- As expected, does not respond to VEGF-B.

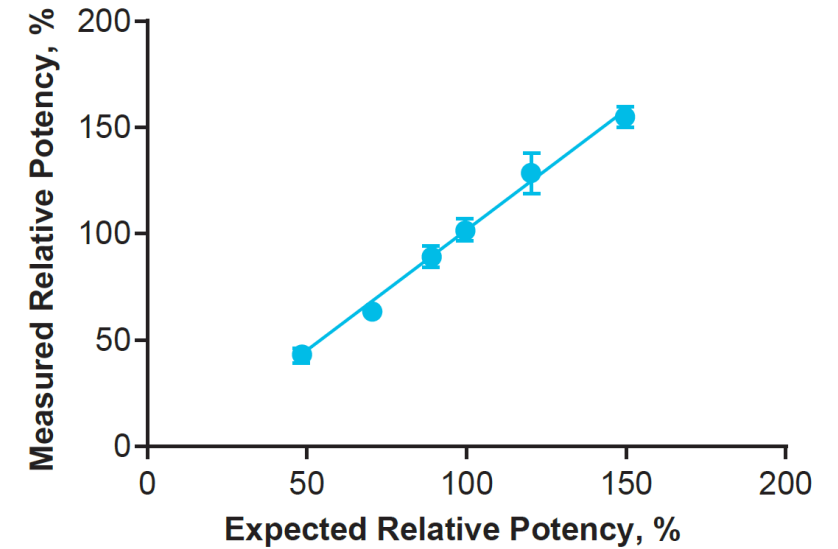
# Bevacizumab Bioassay Response is Highly Stable



Time	EC <sub>50</sub> (ng/mL)	S/B Ratio
1 h	4.9	3.6
2 h	4.84	4.0
3 h	4.60	4.2
4 h	4.9	4.3
5 h	5.38	4.3
24 h	4.99	4.6

## Potency, Linearity, Accuracy and Precision

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			



**Accuracy: 98.0%**

**Precision: 4.8%**

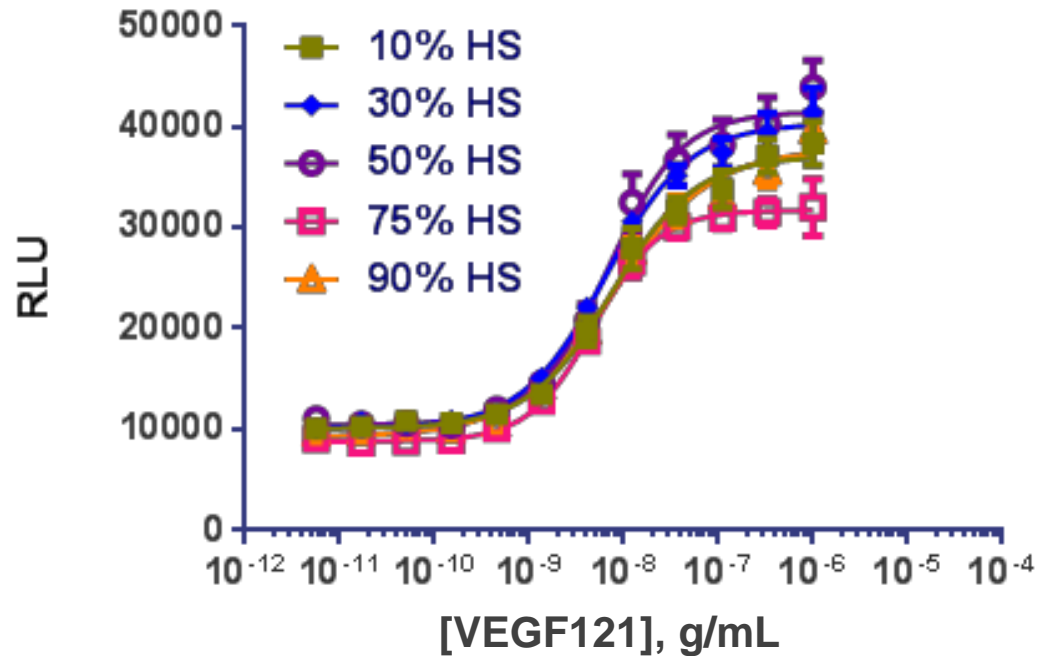
**Linearity: 0.9966**

Bioassay Qualification was done at Covance, UK

Bevacizumab Bioassay Kit

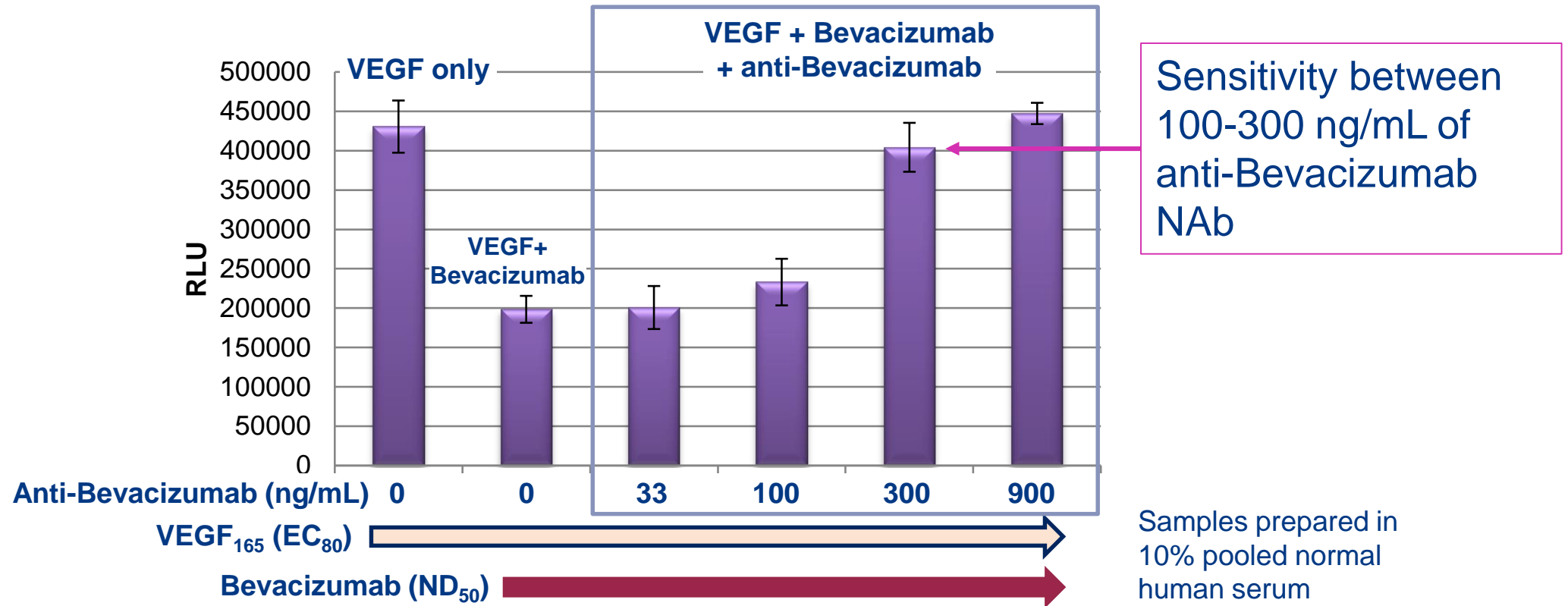
# Application in Anti-Drug Antibody Detection

# Bioassay is highly tolerant to serum



Condition	EC <sub>50</sub> (ng/mL)	S:B Ratio
10% NHS	7.54	3.7
30% NHS	6.51	4.0
50% NHS	6.90	3.9
74% NHS	4.88	3.6
90% NHS	7.81	4.1

# Bevacizumab Bioassay Detects Anti-Drug Antibodies



Note: FDA guidelines specify minimum sensitivity of 1 µg/mL for detection of neutralizing antibodies in human serum

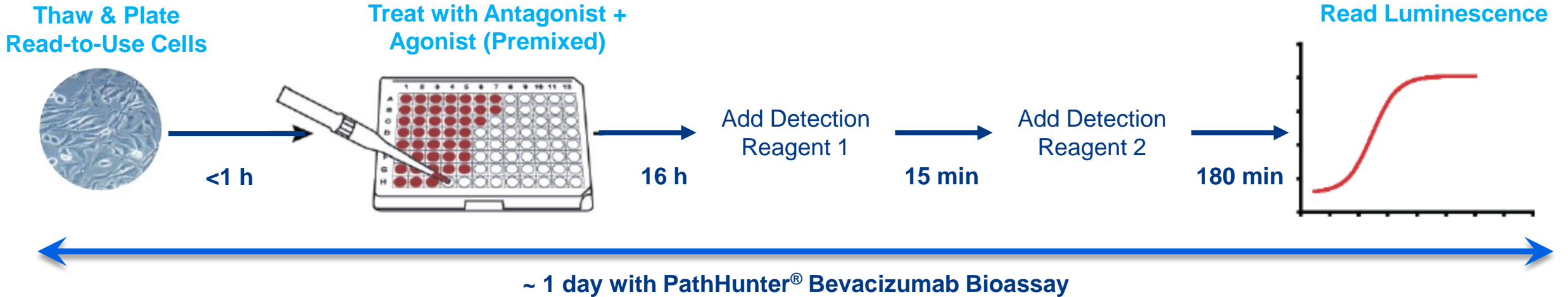
Bevacizumab Bioassay Performance

# Comparison to HUVEC Proliferation Assay



# Simple Bioassay With Results in <24h

*Simple, Homogenous and Rapid Protocol*



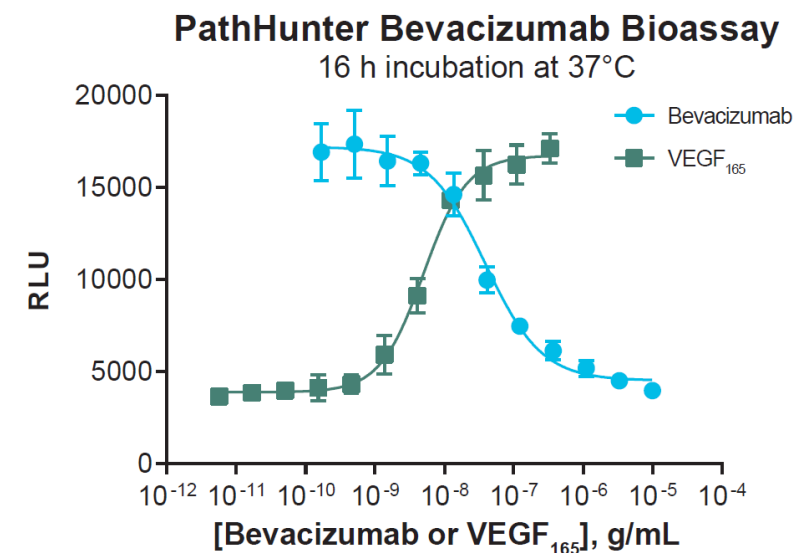
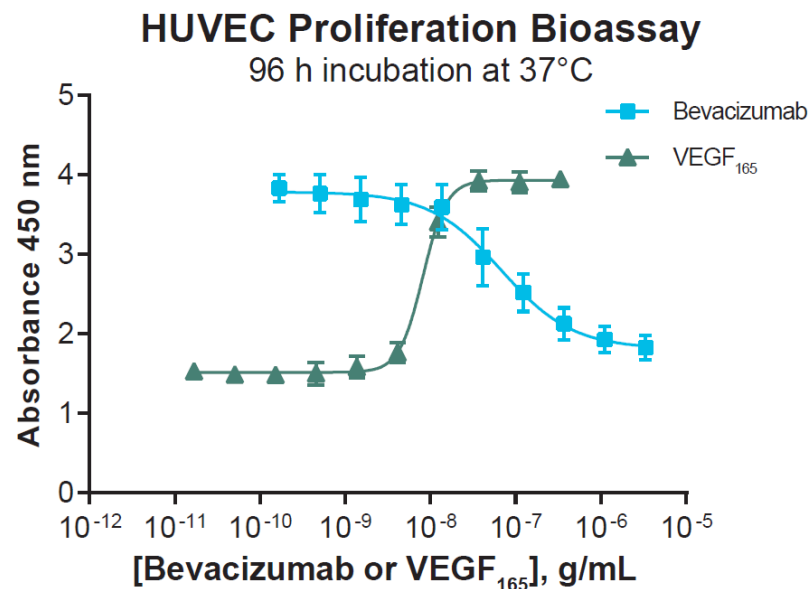
VS.

**2-3 weeks with HUVEC Proliferation Assay**



# Robust Assay Performance

Comparable performance to HUVEC proliferation assay



	HUVEC Proliferation Assay	PathHunter Bevacizumab Bioassay
EC <sub>50</sub> VEGF165	8.2 ng/mL	6.3 ng/mL
EC <sub>50</sub> Bevacizumab	67.9 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
Cell type	Primary cells with donor variability	Clonal, frozen ready-to-assay cells

Sample dataset

# Ranibizumab Bioassay Qualification

# PathHunter® Ranibizumab Bioassay Kit

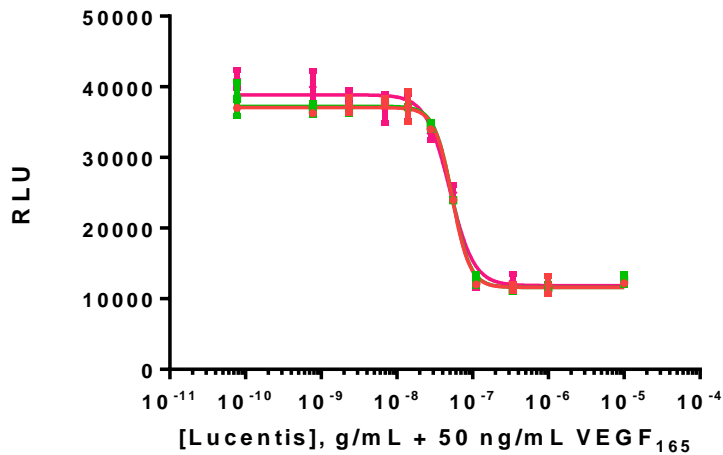
## Kit Components

List of Components	93-0996Y1-00003	93-0996Y1-00004
PathHunter HEK 293 KDR/KDR Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit	200 dp	1,000 dp
Detection Reagent 1	2 mL	10 mL
Detection Reagent 2	8 mL	40 mL
AssayComplete Cell Plating Reagent 0	1 X 100 mL	3 X 100 mL
Protein Dilution Buffer	1 X 50 mL	2 X 50 mL
Control Agonist (VEGF <sub>165</sub> )	1 vial	1 vial
96-Well Opaque-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

# Ranibizumab Bioassay Qualification with Lucentis®

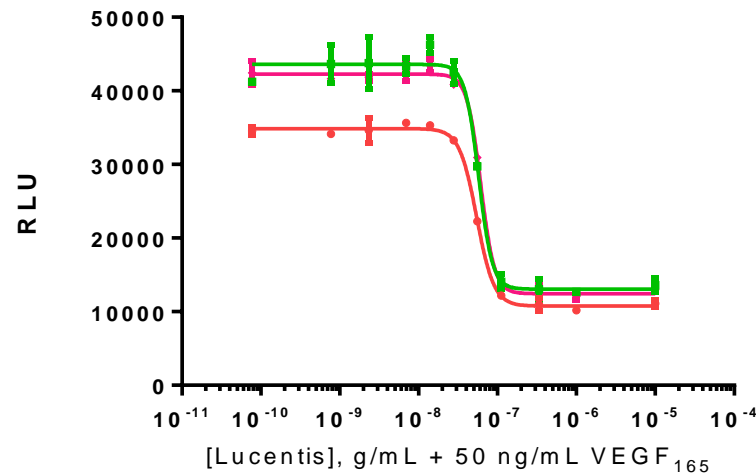
Plate to plate variability: 3 plates with full-plate DRC - Same Day

Plate# 1



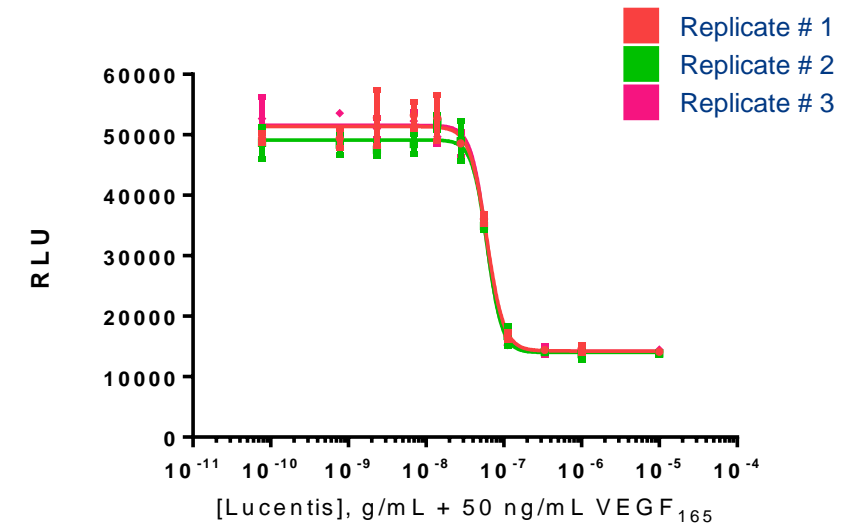
Parameter	R1	R2	R3
S/B	3.1	3.0	3.1
Hill Slope	-3.696	-3.634	-2.744
IC <sub>50</sub> (ng/mL)	53.43	53.59	49.95

Plate# 2



Parameter	R1	R2	R3
S/B	3.1	2.7	2.8
Hill Slope	-3.952	-5.000	-4.969
IC <sub>50</sub> (ng/mL)	54.38	57.56	61.12

Plate# 3



Parameter	R1	R2	R3
S/B	3.4	3.4	3.4
Hill Slope	-3.940	-4.485	-4.352
IC <sub>50</sub> (ng/mL)	60.20	60.87	59.32

## Assay Robustness

### Repeatability and Intermediate Precision (Inter-Plate)

Plate #	Sample	S:B	% RSD, S/B	IC <sub>50</sub> (ng/mL)	Mean IC <sub>50</sub> (ng/mL)	%RSD, IC <sub>50</sub>
1	R1	3.1	3.07	53.4	52.3	3.87
	R2	3		53.6		
	R3	3.1		50		
2	R1	3.1	2.87	54.4	57.7	5.81
	R2	2.7		57.6		
	R3	2.8		61.1		
3	R1	3.4	3.4	60.2	60.1	1.33
	R2	3.4		60.9		
	R3	3.4		59.3		

### Intermediate Precision (Inter-Day)

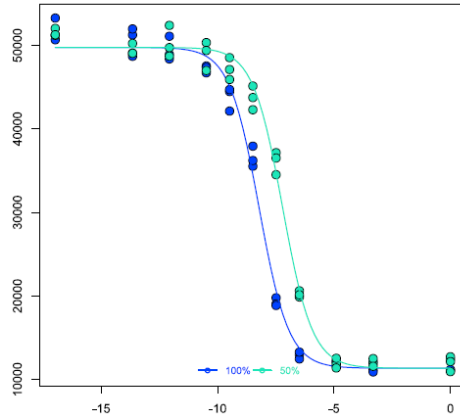
Day	IC50 (ng/mL)	Mean IC50 (ng/mL)	RSD
1	28.0	33.1	7.3
2	35.7		
3	42.2		
4	26.5		

# Ranibizumab Bioassay Qualification with Lucentis®

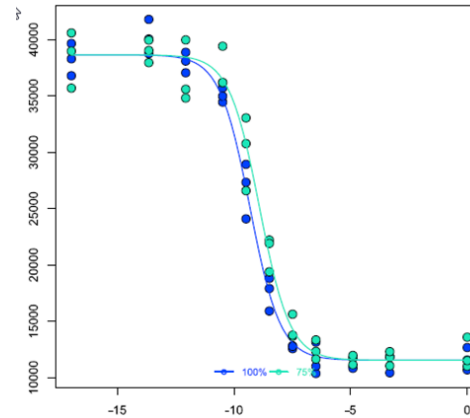
## Parallelism and Potency Estimation (PLA)

Analyst 1

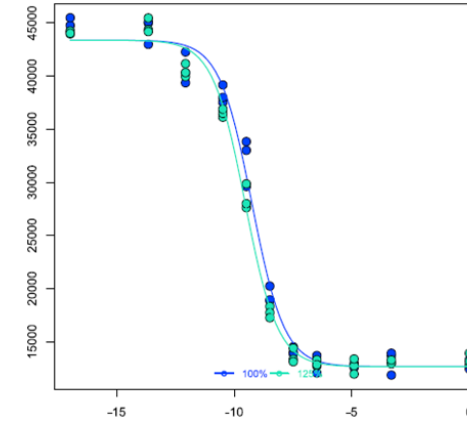
50%



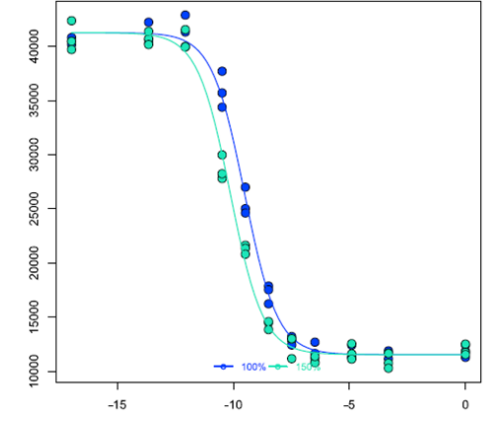
75%



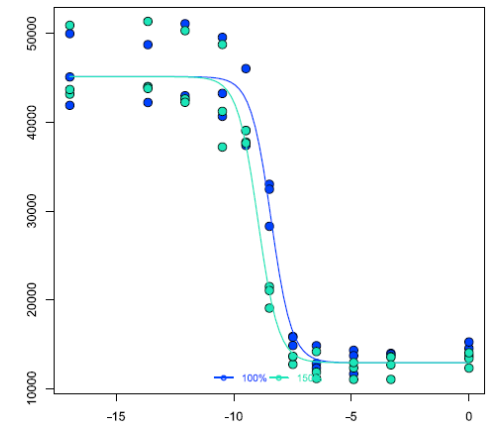
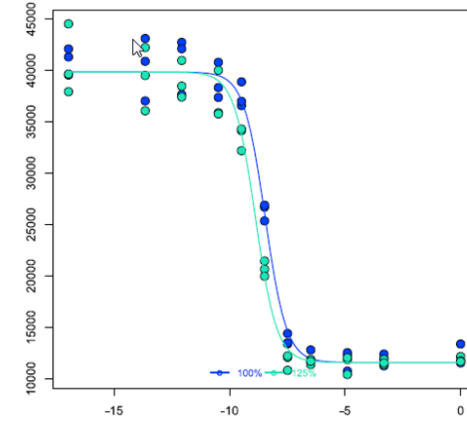
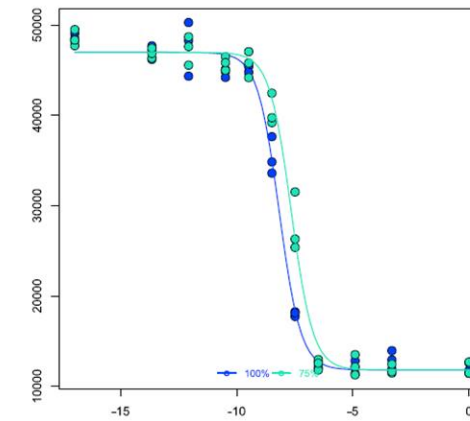
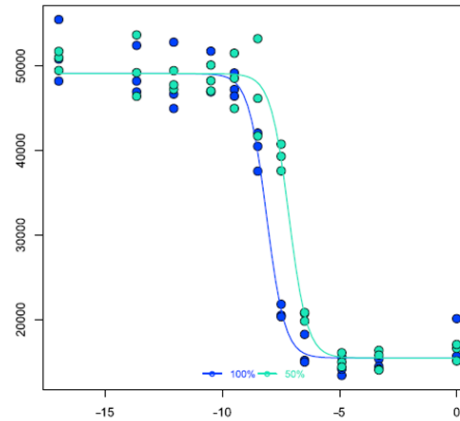
125%



150%



Analyst 2

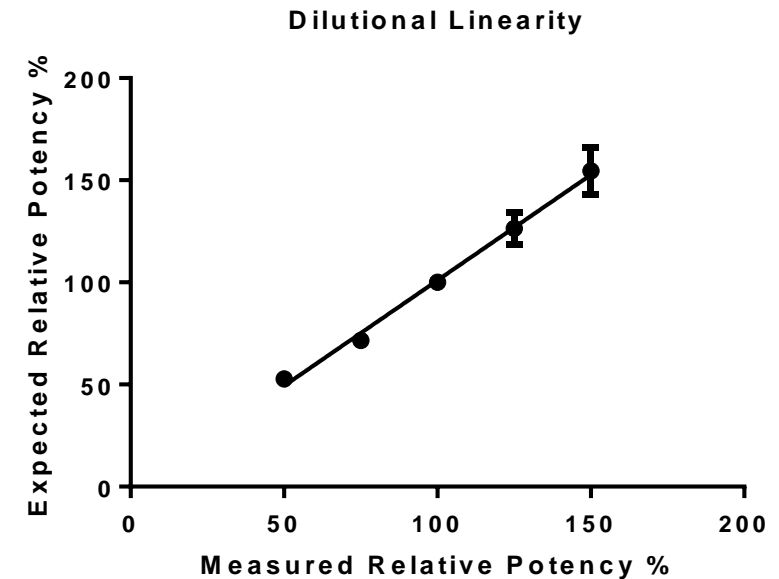


● Reference ● Sample

# Ranibizumab Bioassay Qualification with Lucentis®

Summary: Accuracy, Precision and Dilutional Linearity (2 Analysts)

Expected RP (%)	Exp #	Analyst #	Measured RP (%)	Average RP (%)	% RSD	% Recovery
150	1	1	172.1	154.9	11.9	101.8
	2	1	146.2			
	3	1	153.2			
	4	2	148			
125	1	1	132.2	126.4	8.1	103.2
	2	1	117.2			
	3	1	121.9			
	4	2	134.1			
75	1	1	74.1	71.6	3.7	94.6
	2	1	75.3			
	3	1	67.5			
	4	2	69.6			
50	1	1	57.9	52.7	3.6	104.7
	2	1	51.7			
	3	1	49.5			
	4	2	51.7			



**Accuracy: 100.5%**

**Precision: 5.4%**

**Linearity: 0.9716**



Sample dataset

# Aflibercept Bioassay Qualification

# PathHunter<sup>®</sup> Aflibercept Bioassay Kit

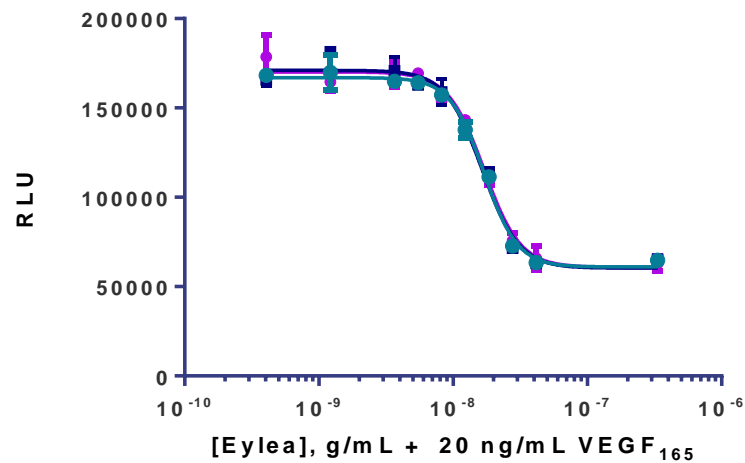
## Kit Components

List of Components	93-0996Y1-00005	93-0996Y1-00006
PathHunter HEK 293 KDR/KDR Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit	200 dp	1,000 dp
Detection Reagent 1	2 mL	10 mL
Detection Reagent 2	8 mL	40 mL
AssayComplete Cell Plating Reagent 0	1 X 100 mL	3 X 100 mL
Protein Dilution Buffer	1 X 50 mL	2 X 50 mL
Control Agonist (VEGF <sub>165</sub> )	1 vial	1 vial
96-Well Opaque-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

# Aflibercept Bioassay Qualification with Eylea®

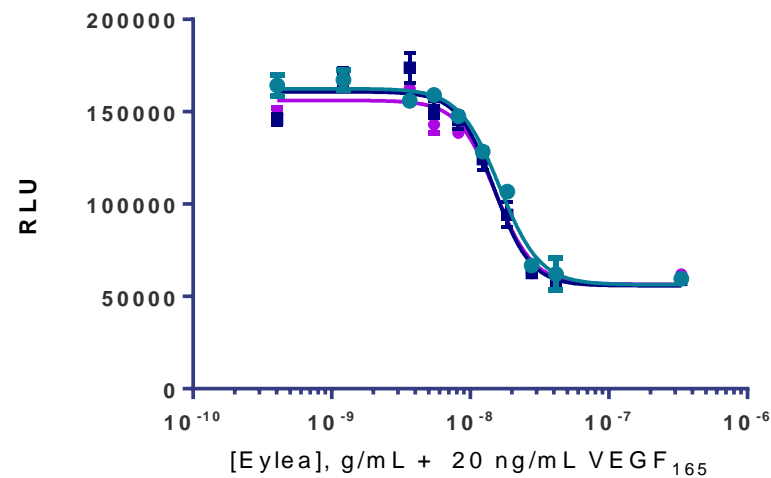
Plate to plate variability: 3 plates with full-plate DRC - Same Day

**Plate# 1**



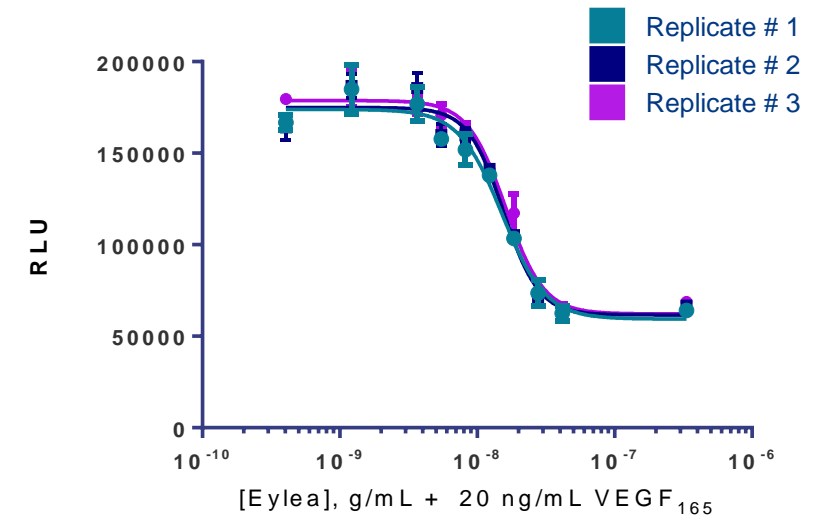
Parameter	R1	R2	R3
S/B	2.9	2.6	2.8
Hill Slope	-3.442	-3.168	-3.218
IC <sub>50</sub> (ng/mL)	16.93	16.43	16.98

**Plate# 2**



Parameter	R1	R2	R3
S/B	2.8	2.5	2.4
Hill Slope	-2.982	-3.301	-3.189
IC <sub>50</sub> (ng/mL)	16.27	14.09	15.20

**Plate# 3**



Parameter	R1	R2	R3
S/B	2.6	2.5	2.6
Hill Slope	-2.752	-3.370	-3.314
IC <sub>50</sub> (ng/mL)	15.18	15.53	16.8

## Assay Robustness

### Repeatability and Intermediate Precision (Inter-Plate)

Plate #	Sample	S:B	% RSD, S/B	IC <sub>50</sub> (ng/mL)	Mean IC <sub>50</sub> (ng/mL)	%RSD, IC <sub>50</sub>
1	R1	2.9	2.8	16.93	16.8	0.3
	R2	2.6		16.43		
	R3	2.8		16.98		
2	R1	2.8	2.6	16.27	15.5	0.7
	R2	2.5		14.9		
	R3	2.4		15.2		
3	R1	2.6	2.6	15.18	15.6	0.5
	R2	2.5		15.52		
	R3	2.6		16.08		

### Intermediate Precision (Inter-Day)

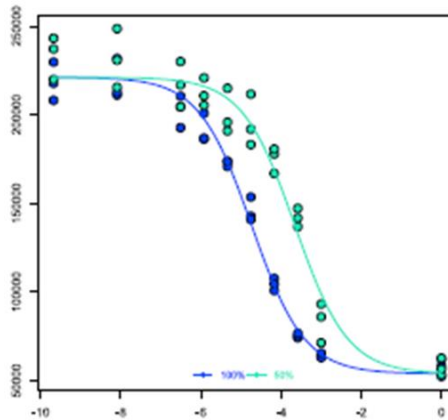
Day	IC <sub>50</sub> (ng/mL)	Mean IC <sub>50</sub> (ng/mL)	%RSD, IC <sub>50</sub>
1	17.6	19.3	4.4
2	24.3		
3	15.9		

# Aflibercept Bioassay Qualification with Eylea<sup>®</sup>

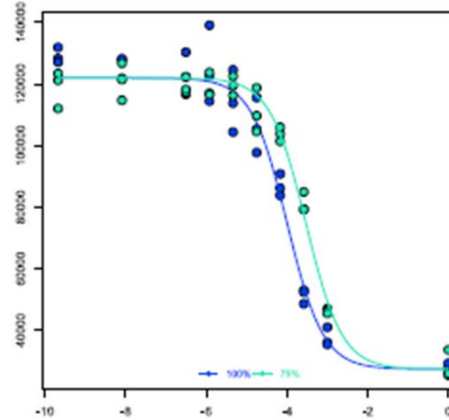
## Parallelism and Potency Estimation (PLA)

Analyst 1

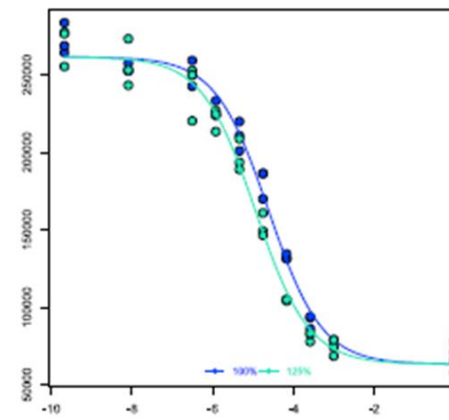
50%



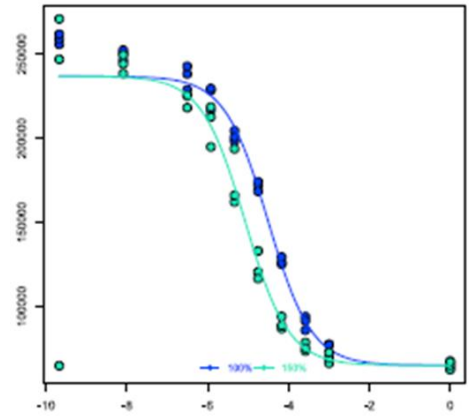
75%



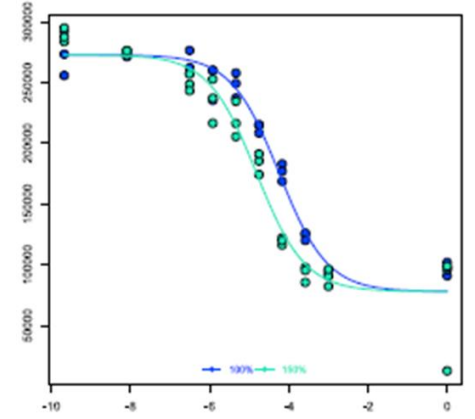
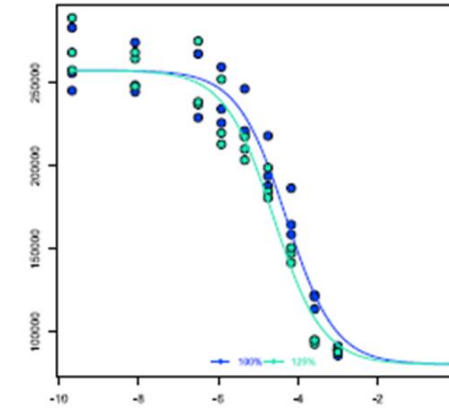
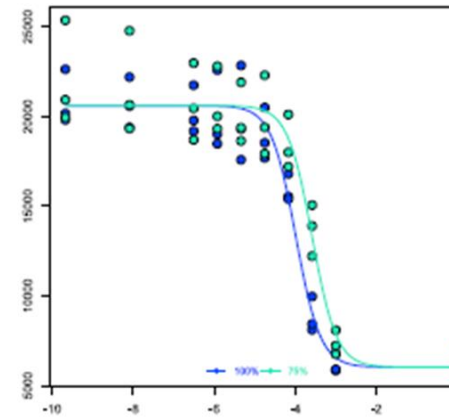
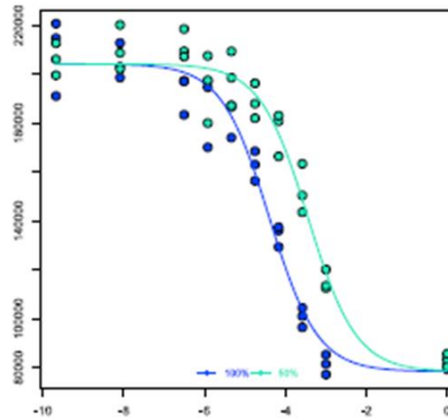
125%



150%



Analyst 2

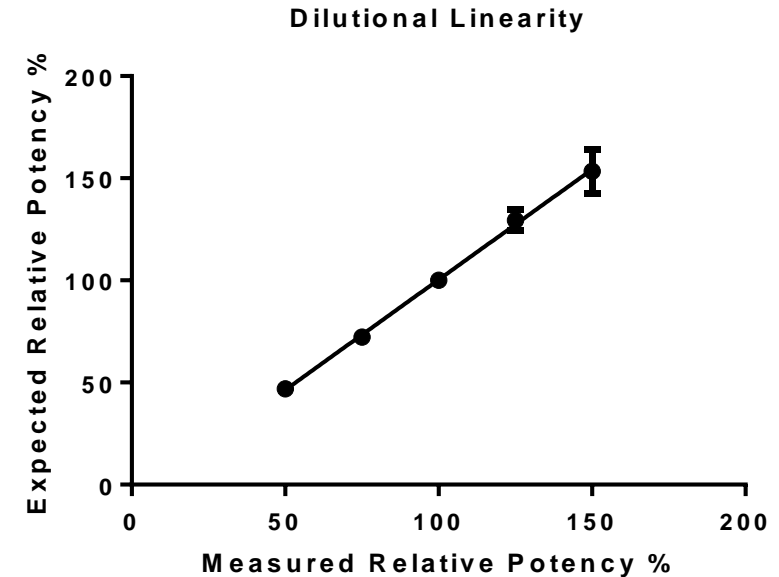


● Reference ● Sample

# Aflibercept Bioassay Qualification with Eylea®

Summary: Accuracy, Precision and Dilutional Linearity (2 Analysts)

Expected RP (%)	Exp #	Analyst #	Measured RP (%)	Average RP (%)	% RSD	% Recovery
150	1	1	147.6	153.4	10.8	101.2
	2	1	169.6			
	3	1	147.6			
	4	2	148.6			
125	1	1	125.5	129.4	5.2	102.2
	2	1	134.8			
	3	1	132.9			
	4	2	124.4			
75	1	1	72.4	72.3	3.0	97.9
	2	1	72.6			
	3	1	68.3			
	4	2	75.7			
50	1	1	47.3	47.0	3.7	97.0
	2	1	42.5			
	3	1	46.6			
	4	2	51.5			



**Accuracy: 96.9%**

**Precision: 3.3%**

**Linearity: 0.9821**

# Benefits for “Ready-to-Use” Bioassay Kits

Functional response based on drug MOA

Verified and Qualified with innovator’s marketed drug

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

High tolerance of matrix components (serum & plasma)

Readily Implement with Optimized kit

- Frozen ready-to-assay cells
- Bioassay Detection Reagents
- Cell Plating Reagent
- Dilution Buffer
- Control Agonist
- Tissue Culture-Treated Plates

# For More Info, Questions or Technical Support



## **Web:**

[Cell-Based Bioassays for Biologics](#)

## **Technical Support**

For NA:

[DRX\\_SupportUS@eurofinsUS.com](mailto:DRX_SupportUS@eurofinsUS.com)

For Europe, Africa & Middle East:

[DRX\\_SupportEurope@eurofinsUS.com](mailto:DRX_SupportEurope@eurofinsUS.com)

For Asia-Pacific:

[AsiaPacificSupport@eurofins.com](mailto:AsiaPacificSupport@eurofins.com)