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PathHunter® Panitumumab Bioassay

Qualified with Vectibix®

93-1051Y3-00093 (2-Plate Kit)
93-1051Y3-00094 (10-Plate Kit)

Panitumumab (Vectibix®)

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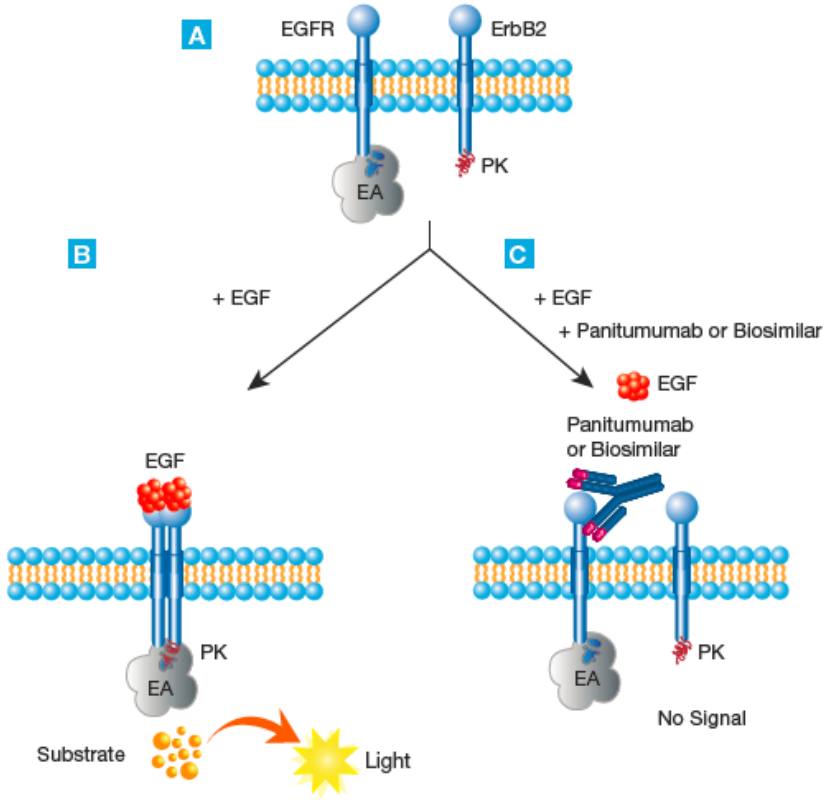
For treatment of EGFR+ metastatic colorectal cancer

Mechanism of Action

EGFR (ErbB1) is known to form homodimers as well as heterodimers with other ErbB family members (e.g. ErbB2, ErbB3) *in vivo*. Heterodimerization with ErbB2 in particular is thought to result in the propagation of malignant signals in many epithelial cancers.

Panitumumab is an IgG2 isotype mAb that acts by binding to the extracellular domain of EGFR preventing its ability to homo-and heterodimerize, therefore inhibiting its activation and subsequent downstream signaling.

Assay Principle



PathHunter® Panitumumab Bioassay Kit

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Kit Components

List of Components	93-1051Y3-00093	93-1051Y3-00094
PathHunter U2OS EGFR/ErbB2 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 5	1 X 100 mL	3 X 100 mL
Protein Dilution Buffer	1 X 50 mL	2 X 50 mL
Control Agonist (EGF)	1 vial	1 vial
96-Well Clear-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

Sample data

Panitumumab Bioassay Qualification

Bioassay Workflow

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Simple, Homogenous and Rapid Protocol

Plate Read-to-Use Cells **Treat with Agonist/ Molecule** **Read Luminescence**

~ 1.5 days

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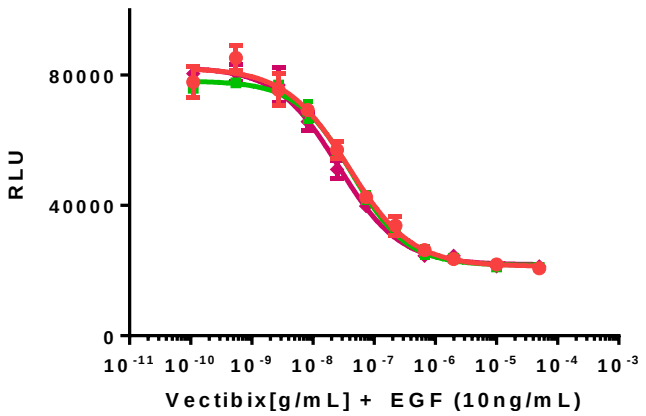
Assay Parameters Assessed

- % 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

Panitumumab Bioassay Qualification with Vectibix®

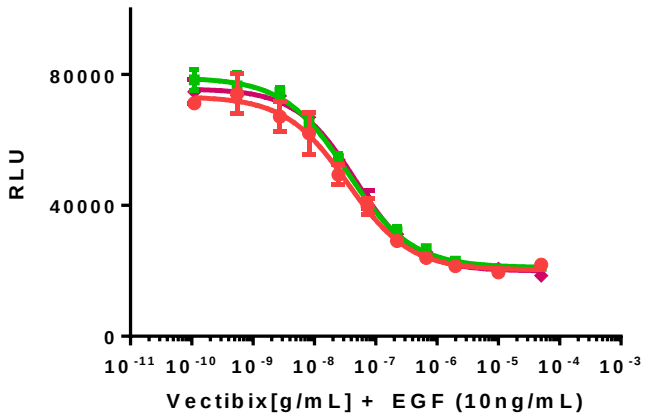
Plate to plate variability: 3 plates with full-plate DRC - Same Day

Plate# 1



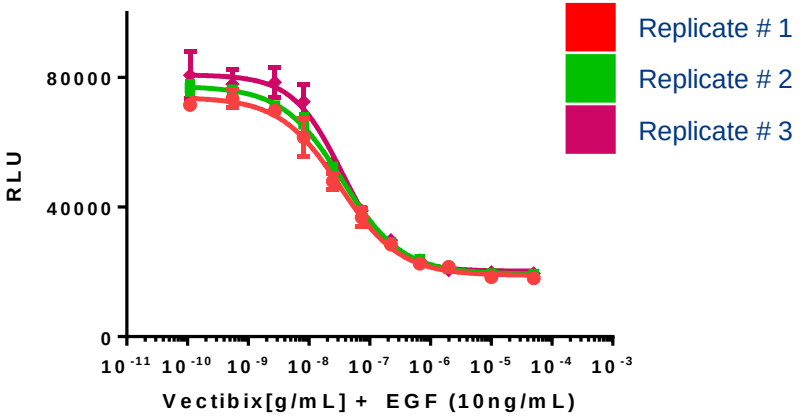
Parameter	R1	R2	R3
S/B	3.9	3.8	4.0
Hill Slope	-0.8437	-0.9570	-0.8616
IC_{50} (ng/mL)	38.2	43.1	25.9

Plate# 2



Parameter	R1	R2	R3
S/B	3.5	3.9	4.1
Hill Slope	-0.8584	-0.8339	-0.8966
IC_{50} (ng/mL)	34.9	34.9	46.4

Plate# 3



Parameter	R1	R2	R3
S/B	4.3	4.3	4.4
Hill Slope	-0.8766	-0.8813	-1.061
IC_{50} (ng/mL)	32.3	35.3	35.2

Panitumumab Bioassay Qualification with Vectibix®

Assay Robustness

Repeatability and Intermediate Precision (Inter-Plate)

Plate	Sample	S/B	%RSD, S/B	IC_{50}, ng/mL	Mean IC_{50}, ng/mL	%RSD, IC_{50}
1	R1	3.9	2.6	38.2	35.7	24.8
	R2	3.8		43.1		
	R3	4		25.9		
2	R1	3.5	8	34.9	38.7	17.1
	R2	3.9		34.9		
	R3	4.1		46.4		
3	R1	4.3	1.3	32.3	34.2	5
	R2	4.3		35.3		
	R3	4.4		35.2		

Intermediate Precision (Inter-Day)

Day	IC_{50}, ng/mL	Mean IC_{50}, ng/mL	%RSD, IC_{50}
1	36.2	42.1	24.2
2	36.2		
3	53.8		

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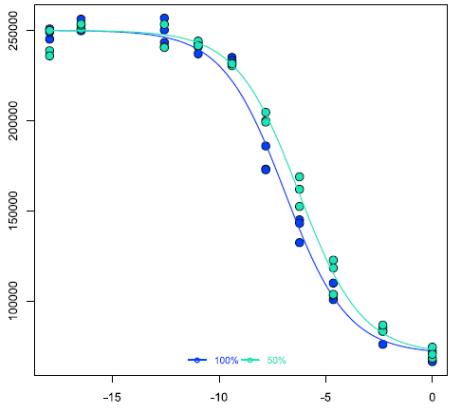
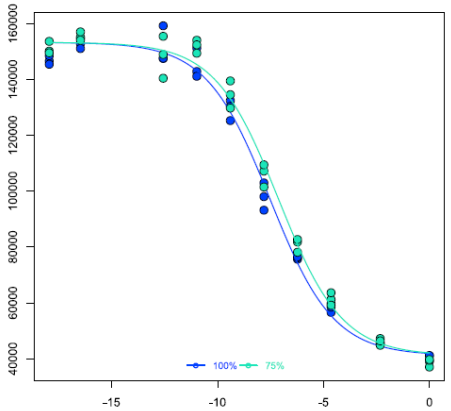
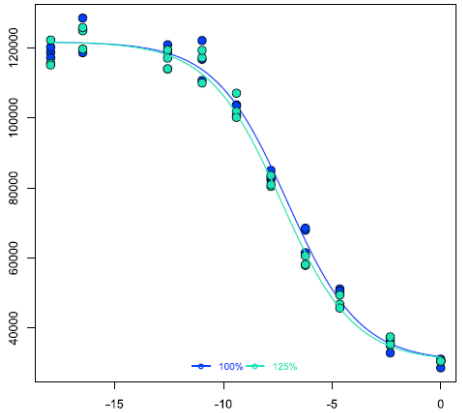
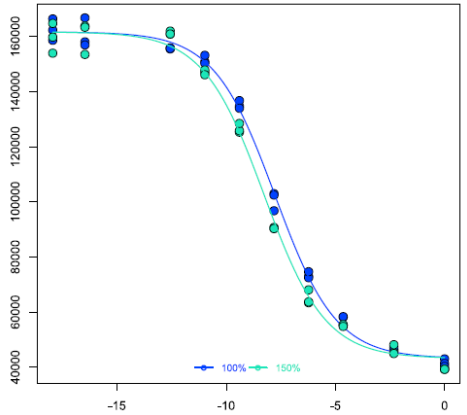
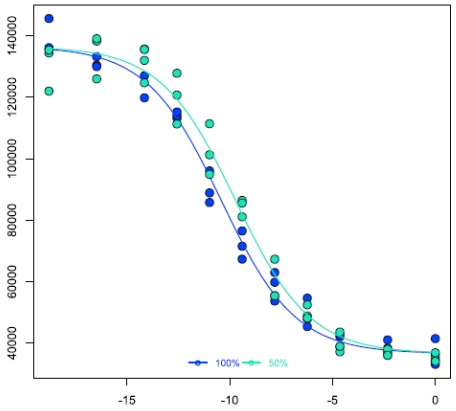
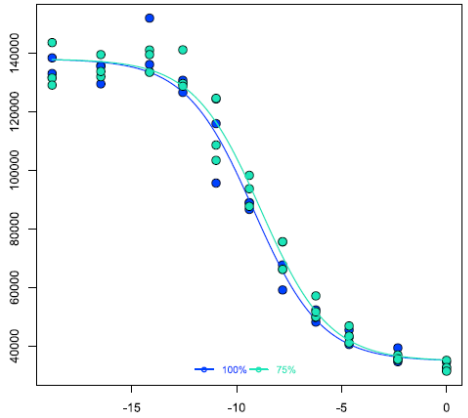
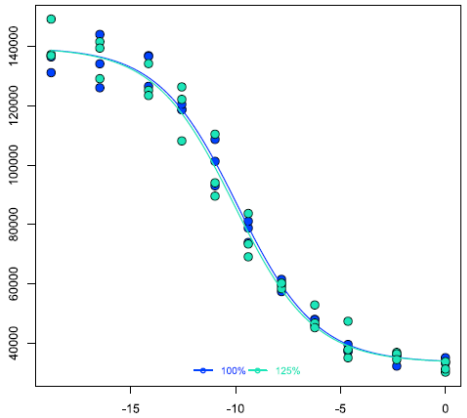
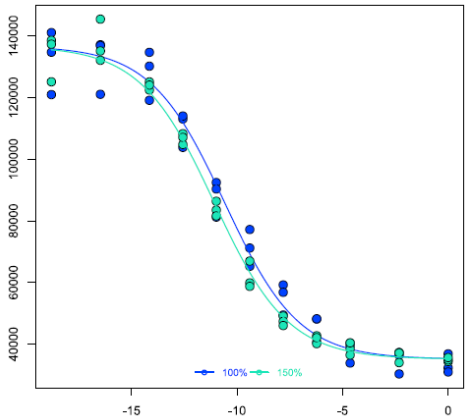
Parallelism and Potency Estimation (PLA)

	50%	75%	125%	150%
Analyst 1				
Analyst 2				

Reference Sample

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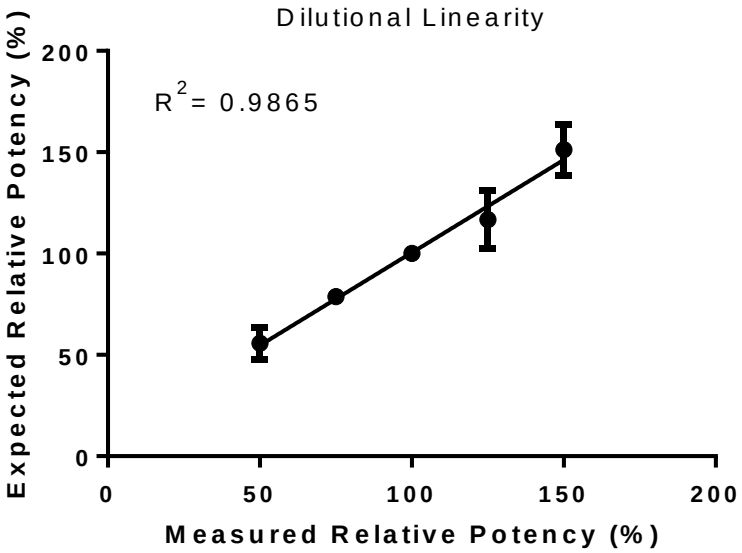
Summary: Accuracy, Precision and Dilutional Linearity (2 Analysts)

Exp#	Analyst #	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1	1	150	140.4	151.3	8.31	100.9
2	1		159.8			
3	1		164.3			
4	2		140.6			
1	1	125	134	116.7	12.14	93.4
2	1		101			
3	1		121.2			
4	2		110.7			
1	1	75	80.7	78.8	2.89	105.1
2	1		75.7			
3	1		80.3			
4	2		78.5			
1	1	50	63.6	55.7	14.6	110
2	1		49.3			
3	1		61.8			
4	2		48.1			



Accuracy: 102.4%

Precision: 9.48%

Benefits for “Ready-to-Use” Bioassay Kits

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Functional response based on drug MOA

Verified and Qualified with innovator drug

Simple protocol; Rapid results

Specific and Sensitive assay

Highly reproducible

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

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