PathHunter® Pertuzumab Bioassay

Qualified with Perjeta®

93-1042Y3-00095 (2-Plate Kit)

93-1042Y3-00096 (10-Plate Kit)

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## For Treatment of HER2+ breast cancer

Mechanism of Action

HER2/ErbB2 is known to form homodimers as well as heterodimers with other ErbB family members (e.g. ErbB2, ErbB3) *in vivo*.

Heterodimerization with ErbB3 is the most potent combination for activating signaling pathways that lead to dysregulated growth / cell proliferation.

Pertuzumab is an IgG1 isotype mAb that acts by binding to the domain on ErbB2 that interacts with ErbB3, thus inhibiting the dimerization of ErbB2 with ErbB3, resulting in inhibition of downstream signaling.

Assay Principle
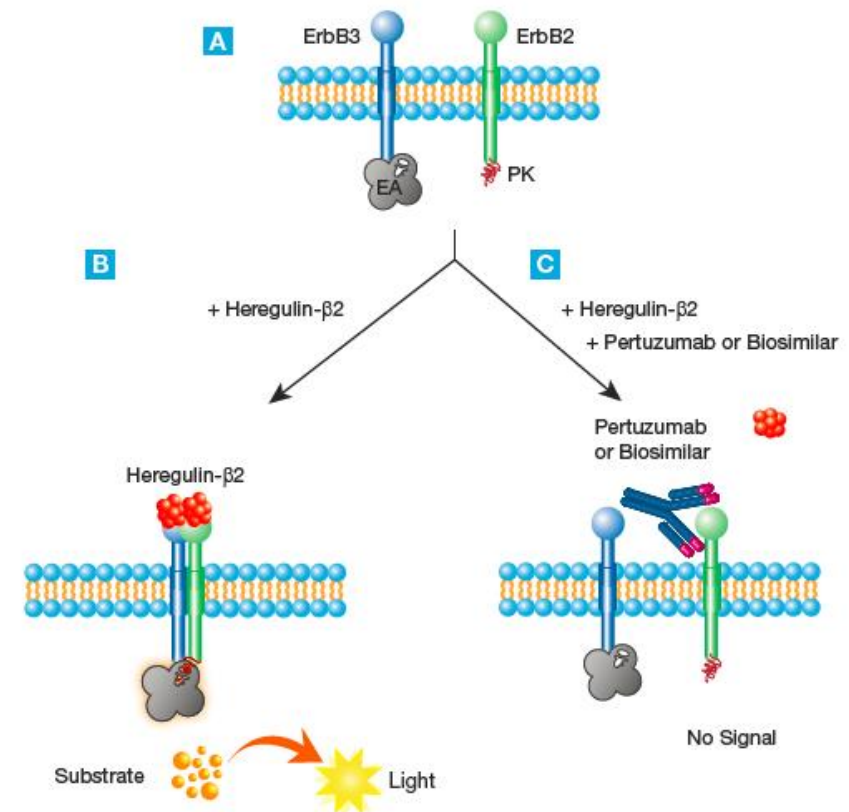
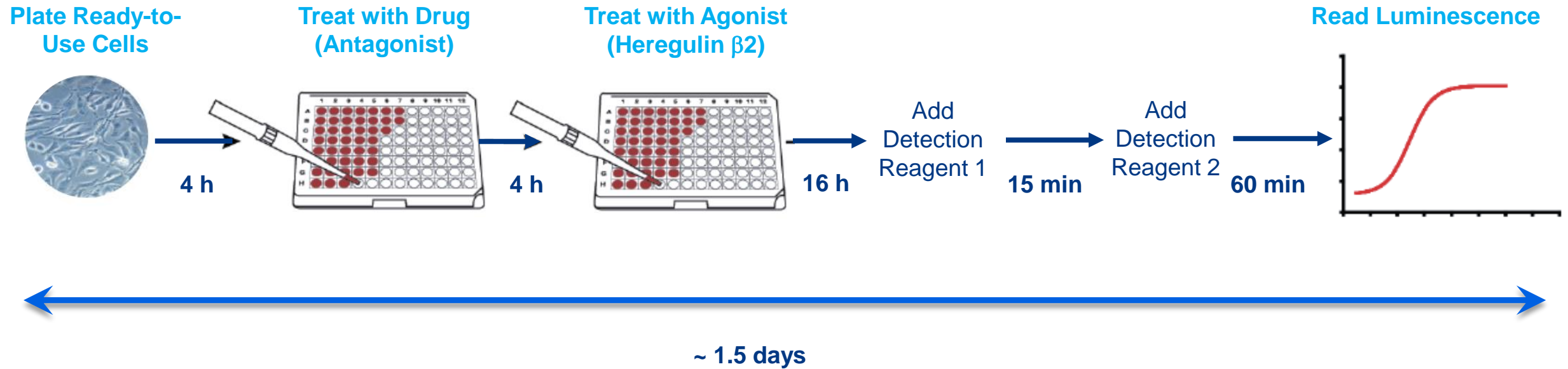# PathHunter® Pertuzumab Bioassay Kit

Kit Components

List of Components	93-1042Y3-00095	93-1042Y3-00096
PathHunter U2OS ErbB2/ErbB3 Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit		
Detection Reagent 1 (mL)	2	10
Detection Reagent 2 (mL)	8	40
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 (Heregulin-β2)	1 vial	1 vial
96-Well Clear-Bottom TC Treated, Sterile Plates w/Lid	2 plates	10 plates

Sample data

Pertuzumab Bioassay Qualification

Bioassay Workflow

*Simple, Homogenous and Rapid Protocol*

Plate Ready-to-Use Cells → **4 h** → **Treat with Drug (Antagonist)** → **4 h** → **Treat with Agonist (Heregulin β2)** → **16 h** → Add Detection Reagent 1 → **15 min** → Add Detection Reagent 2 → **60 min** → **Read Luminescence**

~ 1.5 days

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
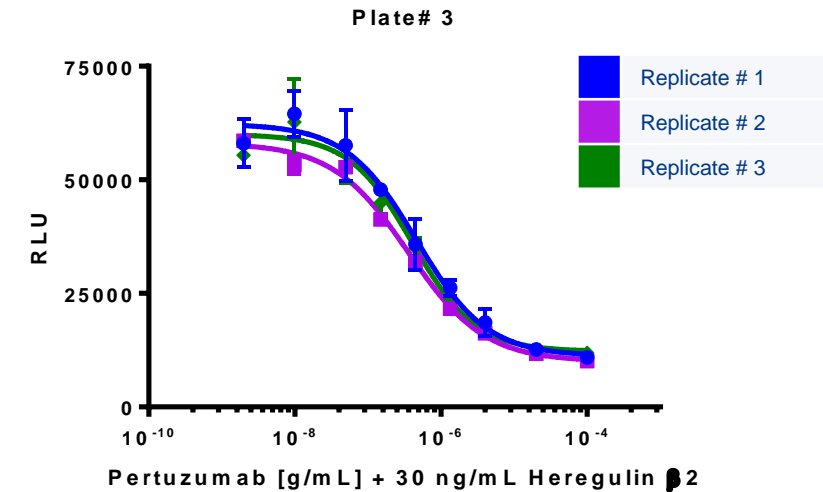
Pertuzumab Bioassay Qualification with Perjeta®

Plate to plate variability: 3 plates with full-plate DRC - Same Day
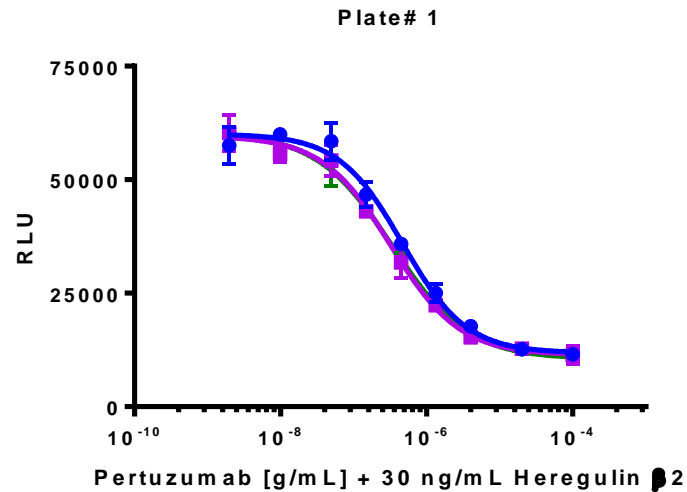
Parameter	R1	R2	R3
S/B	5.2	5.3	5.7
Hill Slope	-0.9952	-0.9197	-0.8406
IC$_{50}$ (ng/mL)	466.6	323.1	335.8
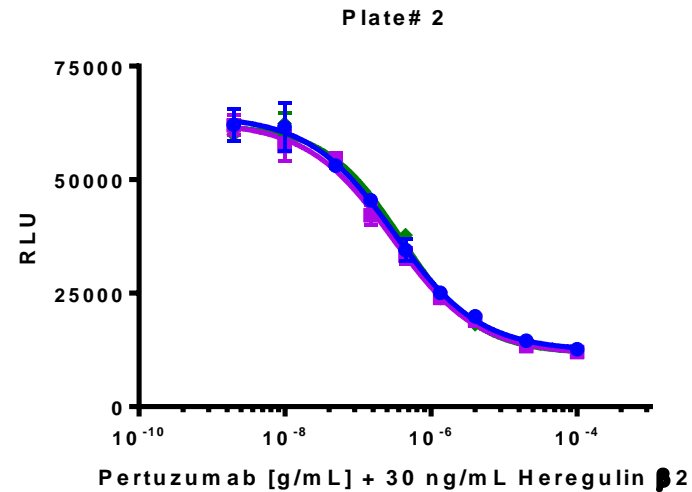
Parameter	R1	R2	R3
S/B	5.1	5.1	5.3
Hill Slope	-0.7423	-0.7404	-0.8315
IC$_{50}$ (ng/mL)	312	295.4	397.4

Parameter	R1	R2	R3
S/B	5.7	5.6	6.0
Hill Slope	-0.9016	-0.8358	-0.9993
IC$_{50}$ (ng/mL)	458.7	356.3	405.5

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Assay Robustness

Intra-day Precision

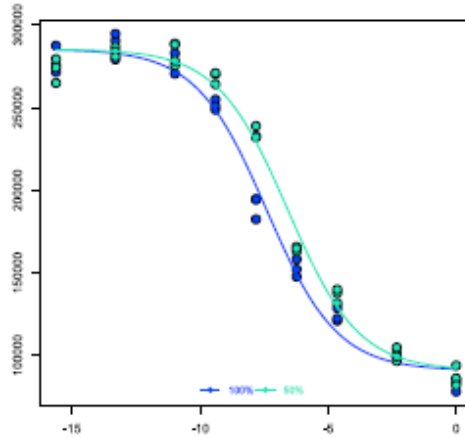| Plate #	Sample	S/B	%RSD, S/B	IC_{50} (ng/mL)	Mean IC50 (ng/mL)	%RSD IC_{50}
1	R1	5.2	5%	466.6	375.2	21%
	R2	5.3		323.1		
	R3	5.7		335.8		
2	R1	5.1	2%	312	334.9	16%
	R2	5.1		295.4		
	R3	5.3		397.4		
3	R1	5.7	4%	458.7	406.9	13%
	R2	5.6		356.3		
	R3	6		405.6		

Inter-day Precision

Day	IC_{50} (ng/mL)	Mean IC_{50} (ng/mL)	%RSD
1	453	474	24%
2	597		
3	372		

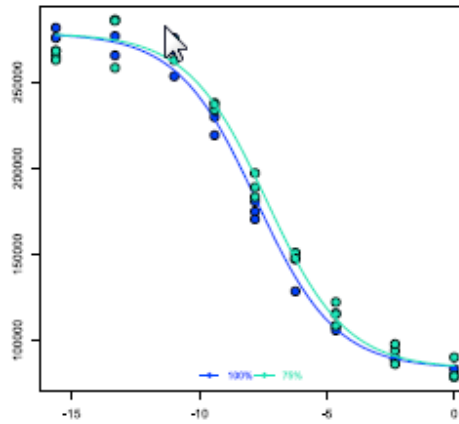# Pertuzumab Bioassay Qualification with Perjeta®

Parallelism and Potency Estimation (PLA)

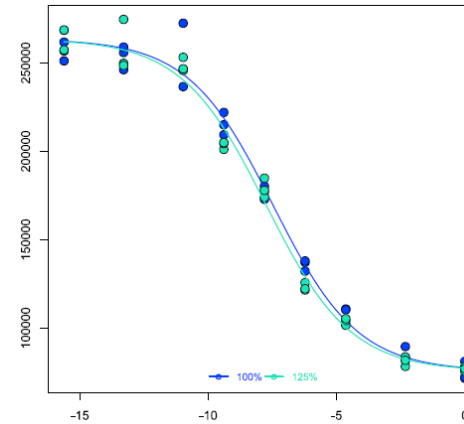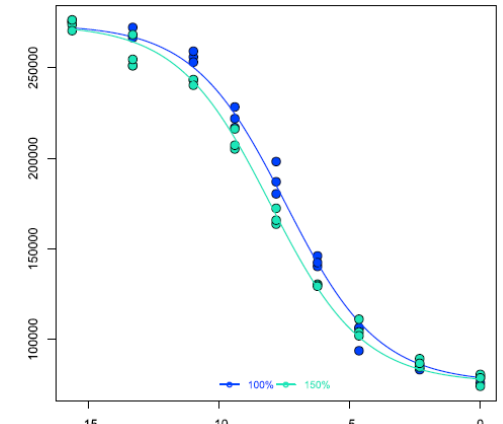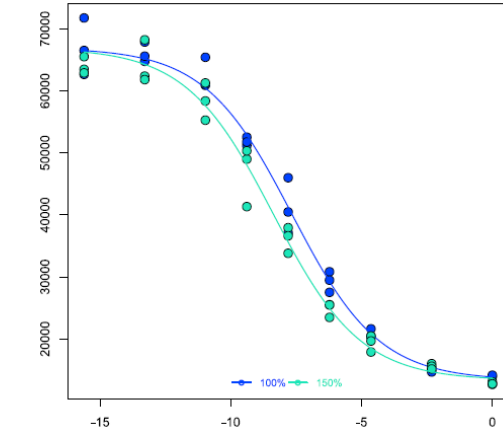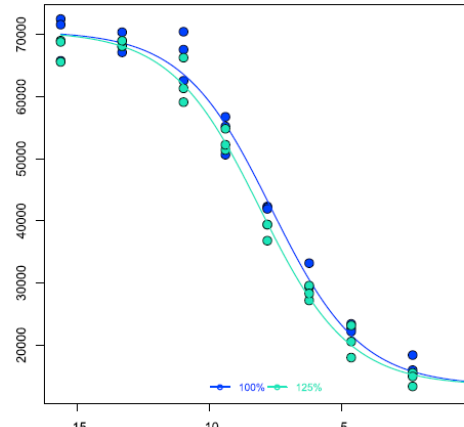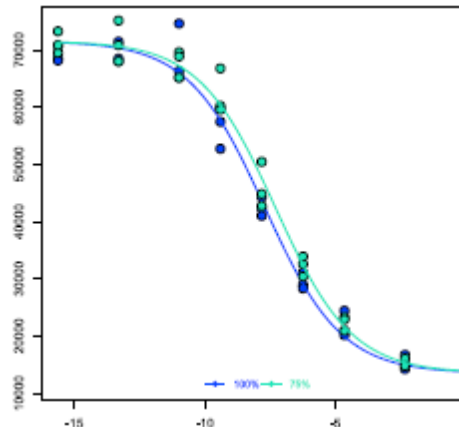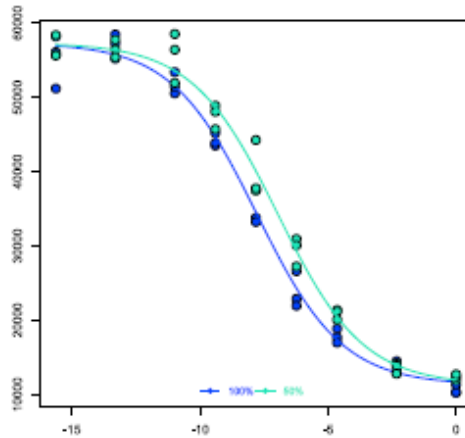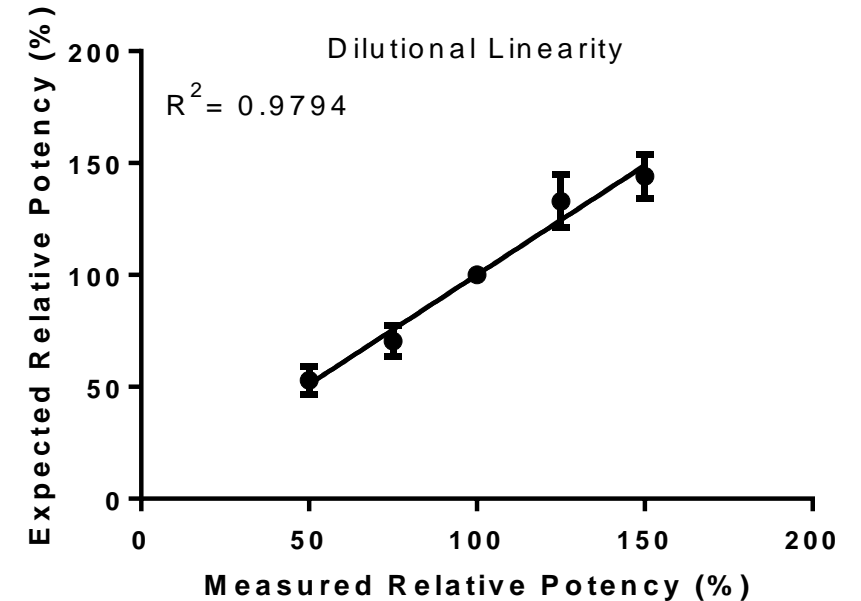Legend: Reference, Sample

Pertuzumab Bioassay Qualification with Perjeta®

Summary: Accuracy, Precision and Dilutional Linearity

Exp#	Expected RP (%)	Observed RP (%)	Average RP (%)	% RSD	% Recovery
1		132.6			
2	150	148.9	144	6.9	96
3		150.6			
1		134.6			
2	125	120.3	132	8.98	105.6
3		144			
1		62.6			
2	75	75.8	70.4	9.8	93.9
3		72.8			
1		56.8			
2	50	45.8	53	11.7	106
3		56.3			

Dilutional Linearity

$R^2 = 0.9794$

Accuracy: 100.4%

Precision: 9.35%

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

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

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