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PathHunter® Adalimumab Bioassay

Qualified with Humira® (Adalimumab)

93-0538B15-00131 (2-Plate Kit)

93-0538B15-00132 (10-Plate Kit)

Adalimumab-mediated Iκb Degradation

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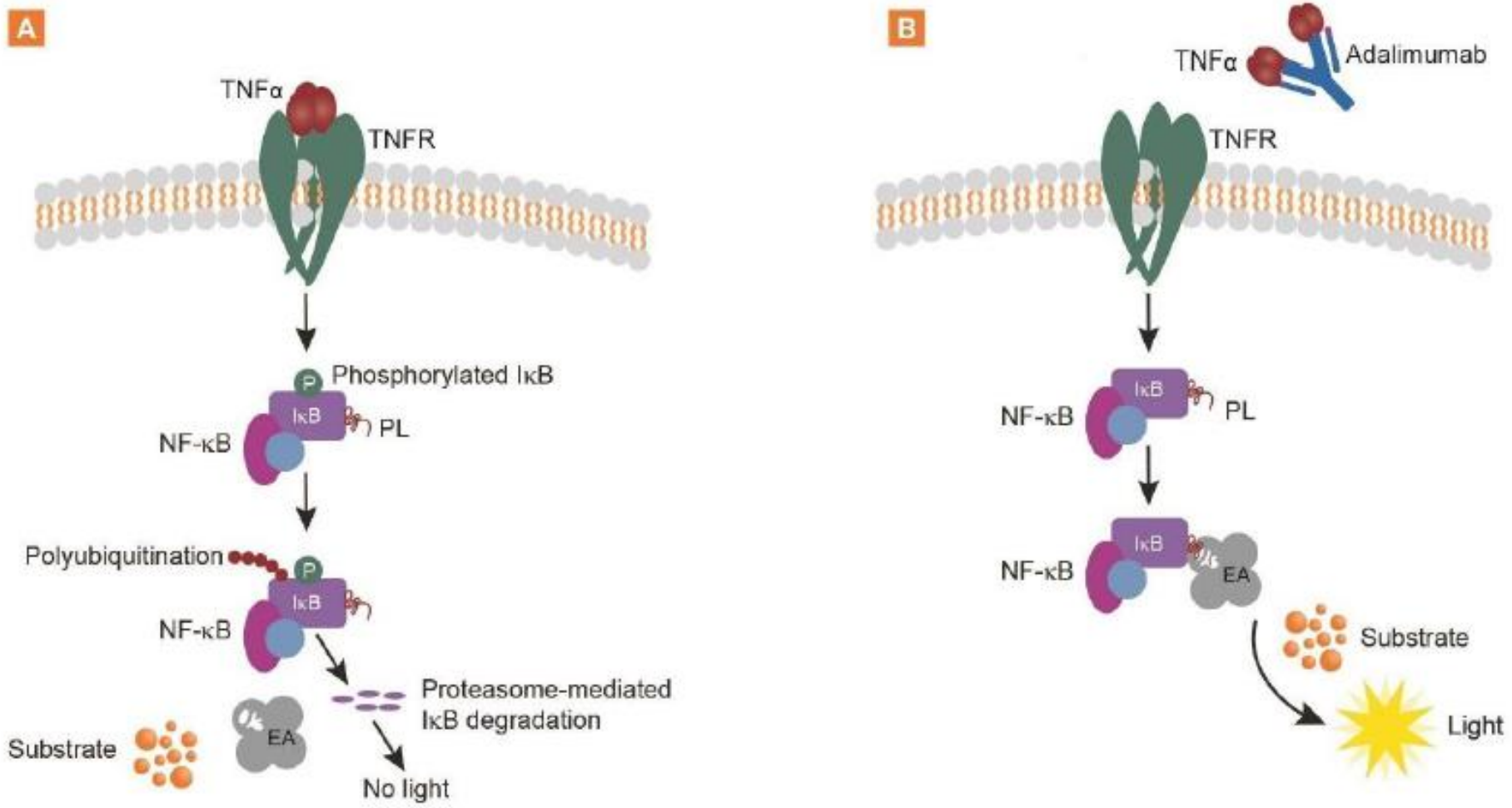
Assay Design



PathHunter® Adalimumab Bioassay Kit

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Kit Components

Materials Provided

List of Components	93-0538B15-00131	93-0538B15-00132
PathHunter A549 IκB Bioassay Cells (1.2 x 10^6 cells in 0.1 mL per vial)	2	10
AssayComplete™ Cell Plating 38 Reagent (100 mL per bottle)	1	2
Recombinant Human TNFα (10 µg per vial)	1	2
AssayComplete Protein Dilution Buffer (50 mL per bottle)	1	2
PathHunter Bioassay ED Detection Kit		
Detection Reagent 1 (Bottle)	1 x 6 mL	1 x 30 mL
Detection Reagent 2 (Bottle)	1 x 20 mL	1 x 100 mL
Detection Reagent 3 (Bottle)	1 x 5 mL	1 x 25 mL
96-Well White, Clear Flat-Bottom, TC-Treated, Sterile Plates with Lid	2	10

Sample Data

Adalimumab Bioassay Kit Qualification

Adalimumab Bioassay Workflow

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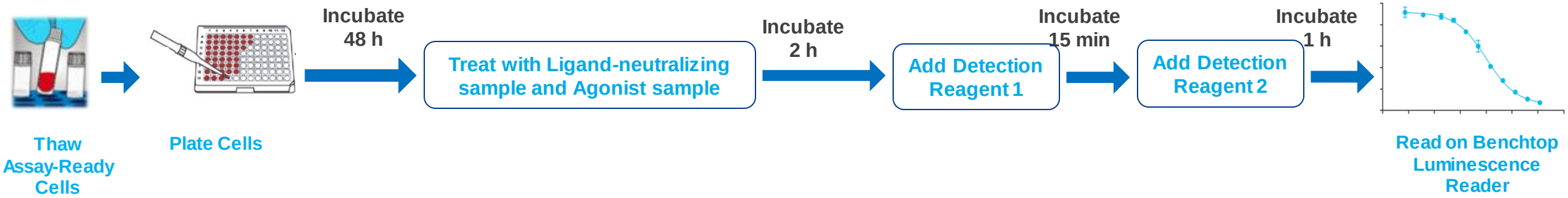
Simple, Homogenous and Rapid Protocol



Total Assay Time: ~ 2 days

Adalimumab Bioassay Qualification with Humira®

Assay Parameters Assessed

- % CV between eight 11-pt DRCs
- Plate uniformity: EC_{80} and IC_{80} (of drug and stimulus) across entire plate
- Plate-to-Plate variability: 3 plates with 11-pt DRCs run on 3 days
- Slope consistency
- Accuracy, precision and linearity of the assay over a range of 50-150% from two operators
  - Assay developer
  - Assay qualifier
- Parallel line analysis

Adalimumab Bioassay Qualification with Humira® Reproducibility Study (Over 3 Days)

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Full Plate

Experiment	Mean S/B	% RSD	Interday Mean S/B	%RSD	Mean IC_{50}, ng/mL	% RSD	Interday Mean IC_{50}, ng/mL	%RSD
Day 1	5.8	4.22			62.72	10.84		
Day 2	5.3	3.03	**5.9**	**11.96**	50.52	6.09	**54.9**	**12.38**
Day 3	6.7	8.10			51.44	4.21		

Minus Rows A and H

Experiment	Mean S/B	% RSD	Interday Mean S/B	%RSD	Mean IC_{50}, ng/mL	% RSD	Interday Mean IC_{50}, ng/mL	%RSD
Day 1	5.8	4.68			64.3	11.31		
Day 2	5.3	3.01	**5.9**	**11.11**	51.2	3.77	**55.7**	**13.3**
Day 3	6.6	3.39			51.7	2.81		

Adalimumab Bioassay Qualification with Humira®
Representative Relative Potency Data (50%-150%)

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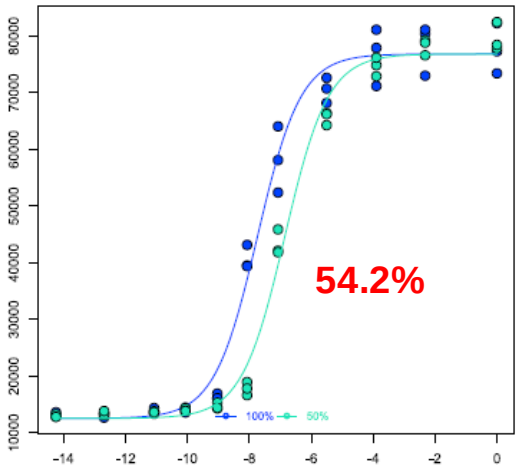
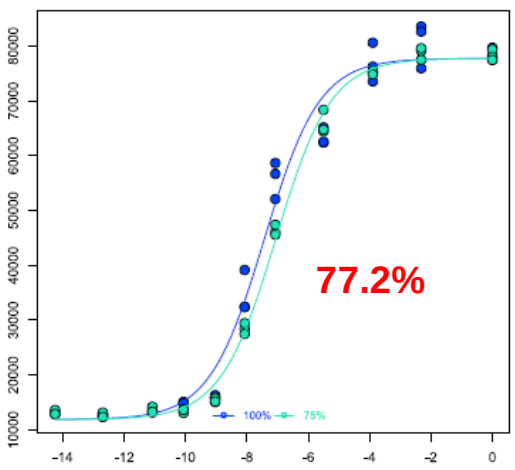
Analyst 1

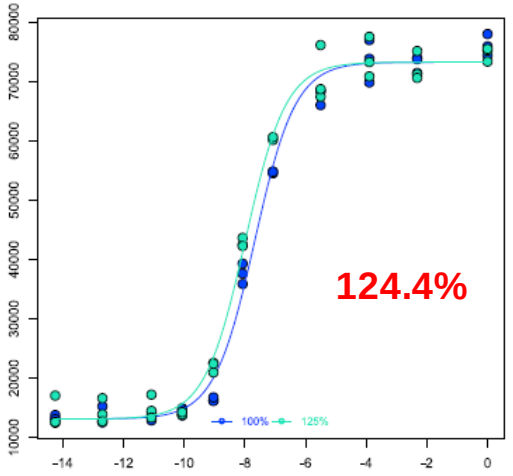
100% vs 50%



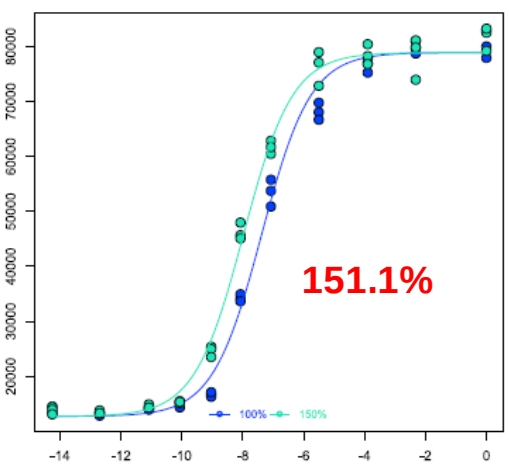
100% vs 75%



100% vs 125%

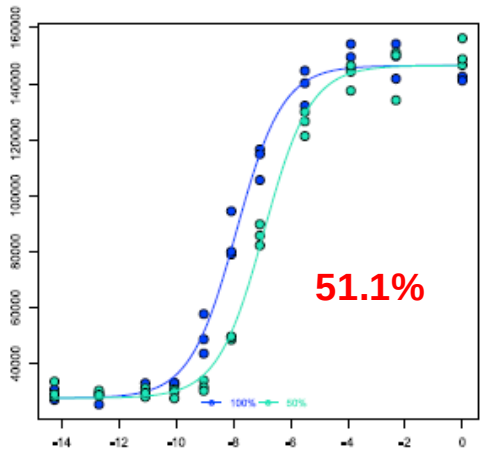


100% vs 150%

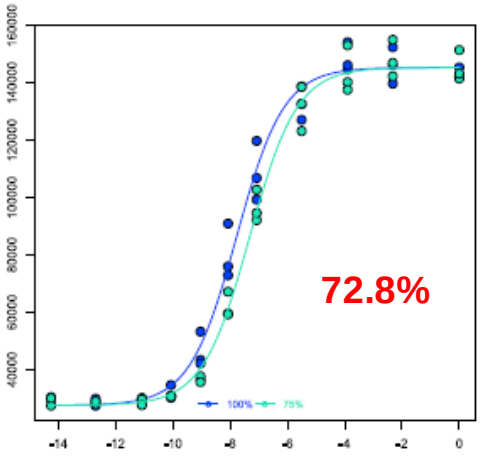


Analyst 2

100% vs 50%



100% vs 75%



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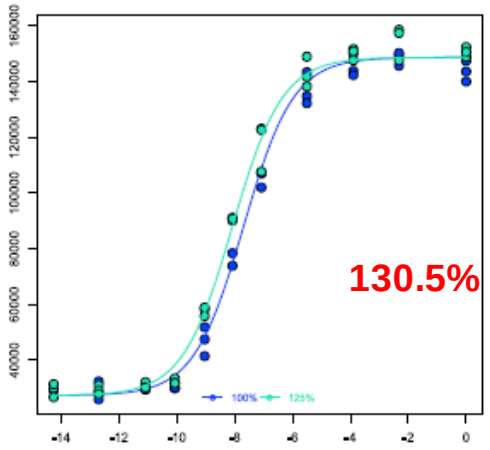
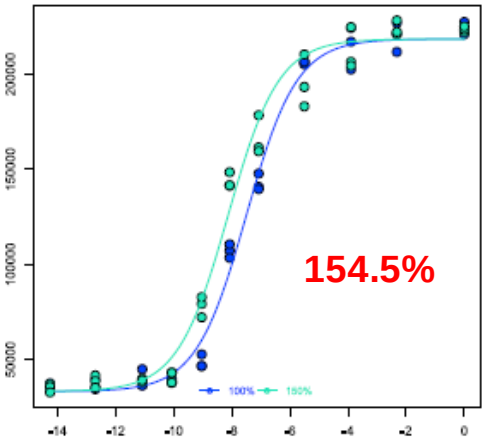


100% vs 150%



Note: RP values calculated in PLA 3.0

Adalimumab Bioassay Qualification with Humira®: Repeatability (Relative Potency)

The ICH Q2B, Section 5.1 recommends a minimum of 6 determinations at 100% nominal potency be used to assess repeatability. Repeatability was assessed using six determinations at 100% nominal relative potency by a single Analyst during the assessment of accuracy in the Adalimumab Bioassay.

Analyst	Observed Potency at 100% Nominal Potency	Average Observed Potency	% RSD
A	88.3%	100.4%	8.44%
	94%		
	107%		
	104.8%		
	110.4%		
	97.9%		
Repeatability Acceptance Criterion:			≤ 20%

Repeatability= 8.4%

Adalimumab Bioassay Qualification with Humira®: Accuracy, Intermediate Precision and Dilutional Linearity

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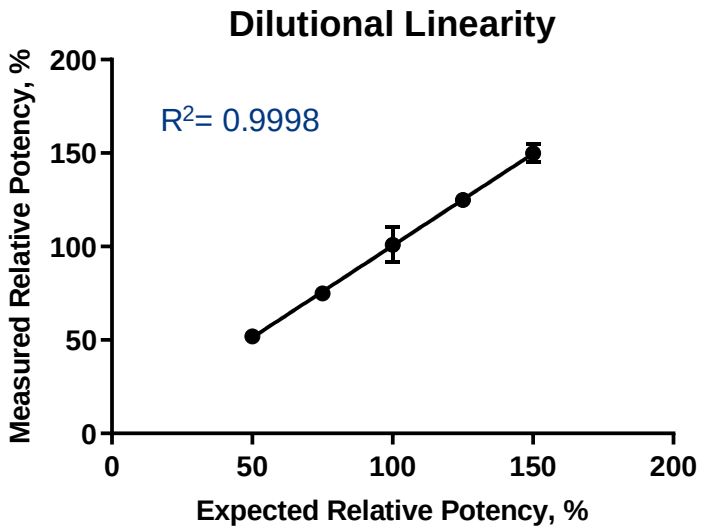
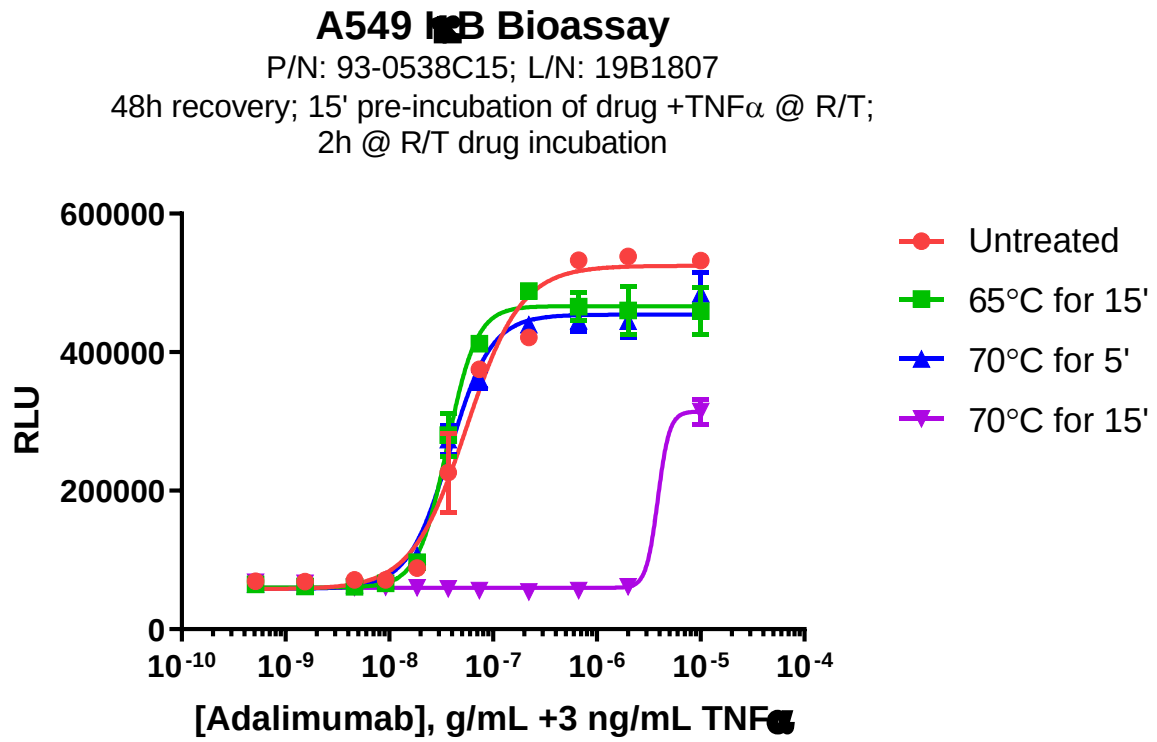
Expected RP (%)	Exp #	Analyst #	Measured RP (%)	Average RP (%)	% RSD	% Recovery
150	1	1	150.6	149.9	3.26	99.9
	2	1	141.6			
	3	1	151.7			
	4	1	151.1			
	5	2	154.5			
125	1	1	126.4	124.9	3.06	99.9
	2	1	122.3			
	3	1	120.7			
	4	1	124.4			
	5	2	130.5			
75	1	1	80	75	4.71	100
	2	1	71.4			
	3	1	73.5			
	4	1	77.2			
	5	2	72.8			
50	1	1	55.5	51.9	5.65	103.8
	2	1	48.4			
	3	1	50.1			
	4	1	54.2			
	5	2	51.1			

Intermediate precision addressed multiple analysts and multiple days



Accuracy= 100.9%
Intermediate precision ≤ 5.65%
Linearity (R^2)= 0.9998

Adalimumab Bioassay Qualification with Humira®
Evaluation of Forced Degradation Samples



	Untreated	65C for 15'	70C for 5'	70C for 15'
Bottom	57786	60377	58409	59813
Top	524711	466425	454184	~ 314258
LogEC50	-7.256	-7.443	-7.419	~ -5.413
HillSlope	1.647	3.066	2.178	~ 7.765
EC50	5.550e-008	3.606e-008	3.810e-008	~ 3.866e-006
S/B	8.0	7.0	7.5	4.5

Treatment of Adalimumab at 70°C for 15 minutes produced a change in potency of the drug outside the range of the assay (right-shifted by two orders of magnitude).

For More Info, Questions or Technical Support

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