



PathHunter® EPO Bioassay Kits

Epoetin alfa:

[93-0965Y3-00018](#) (10-plate)

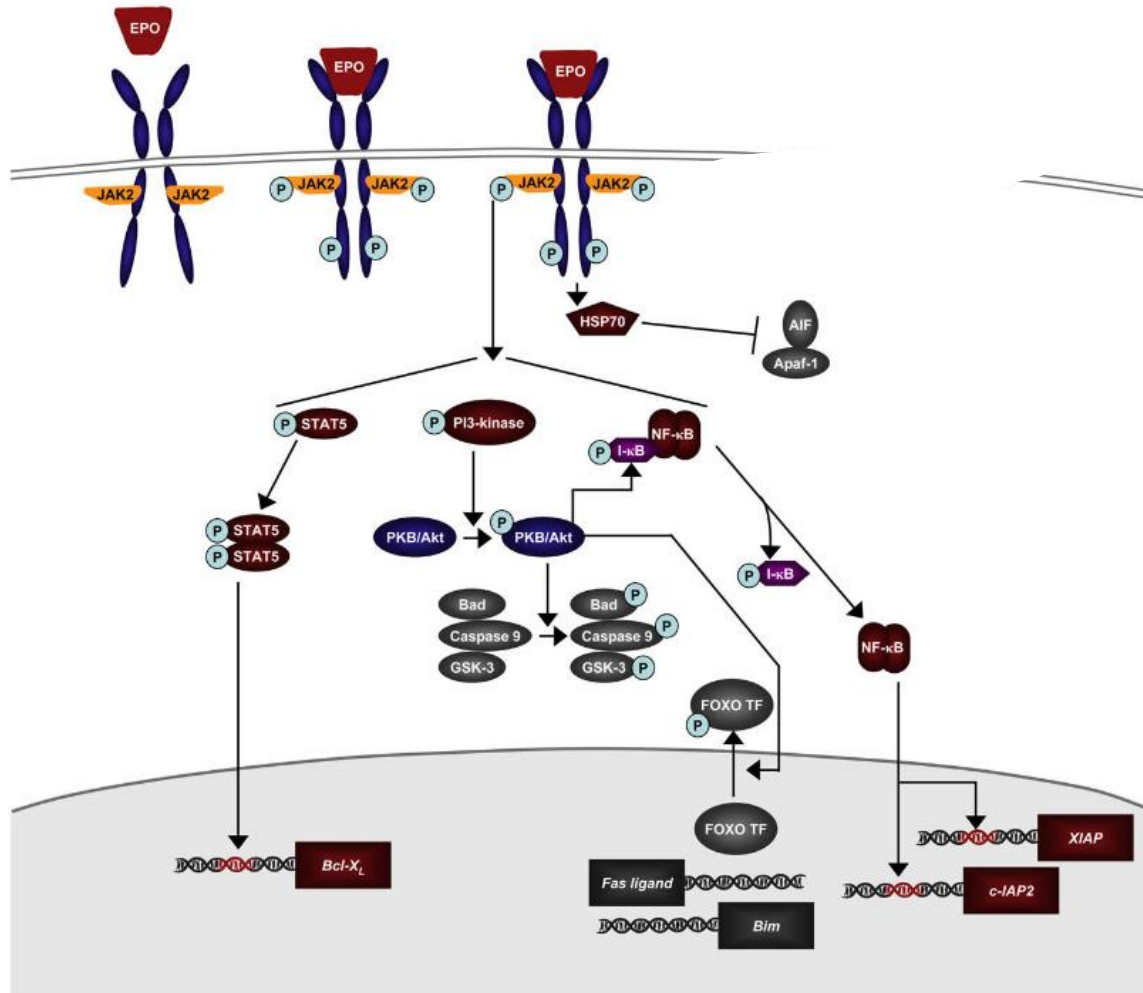
[93-0965Y3-00017](#) (2-plate)

Darbepoetin alfa:

[93-0965Y3-00020](#) (10-plate)

[93-0965Y3-00019](#) (2-plate)

OUR EXPERTISE
IN YOUR HANDS.
DISCOVER
CONFIDENTLY.



S. Elliott et al. / Experimental Hematology 2008;36:1573–1584

Existing assays

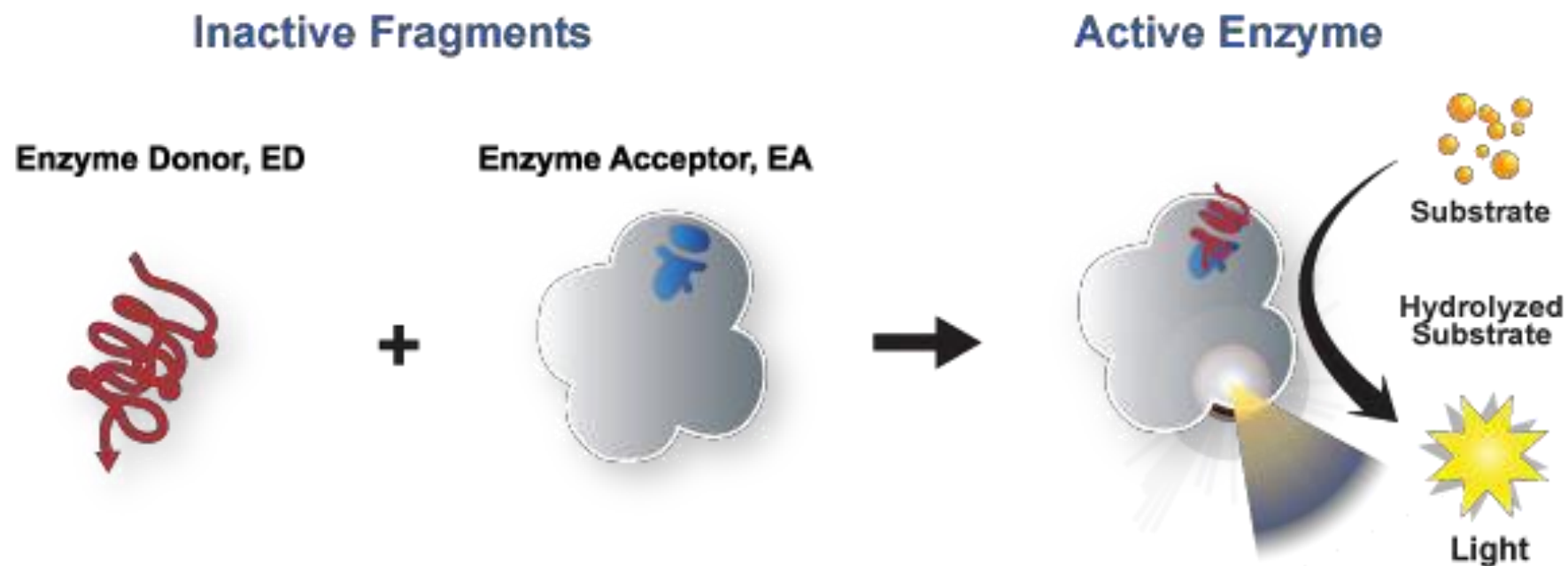
1. TF-1 Proliferation assay

- 72-96 hours incubation times
- Low S:B ratio
- Cell culture required

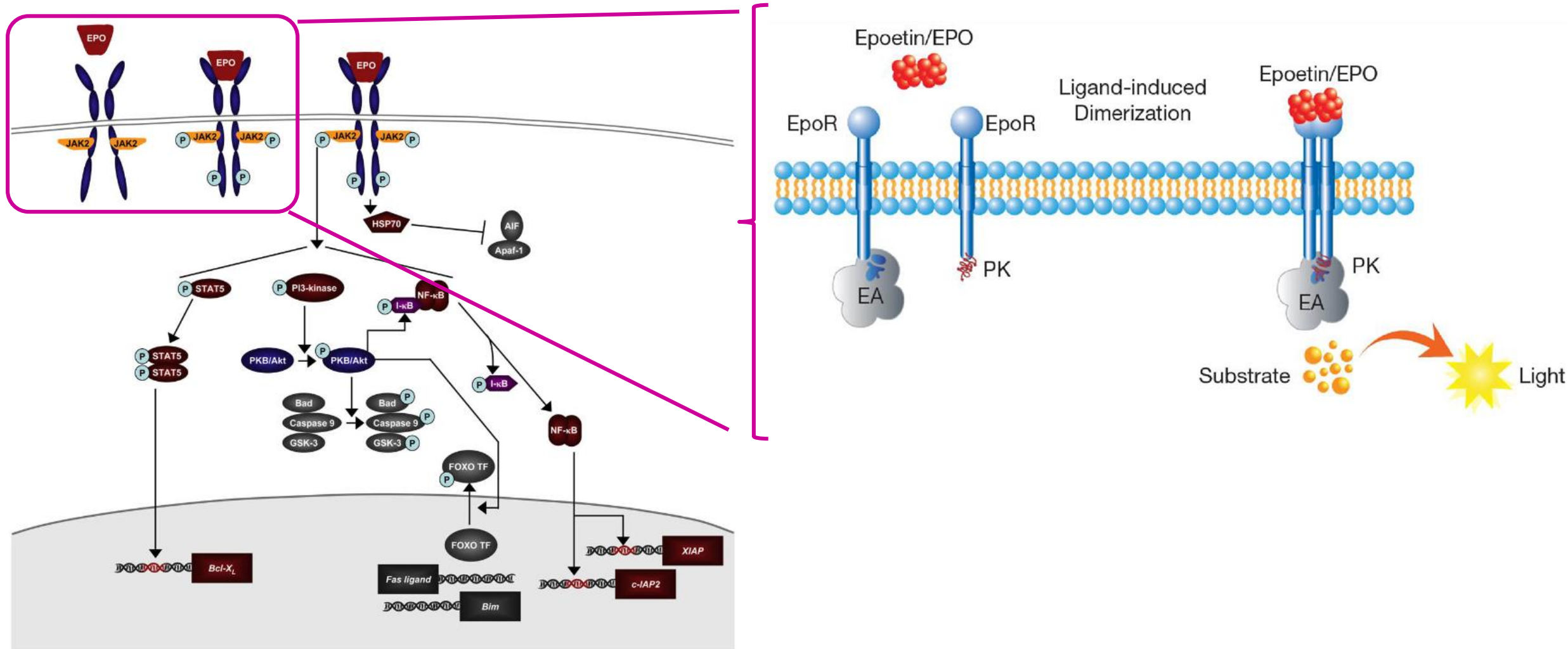
2. Mouse RBC production

- Animal-based assay
- High cost & variability

PathHunter Enzyme Fragment Complementation Technology



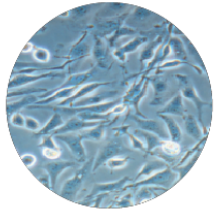
PathHunter EPO Dimerization Assay Principle



Simple, Homogenous & Rapid Protocol

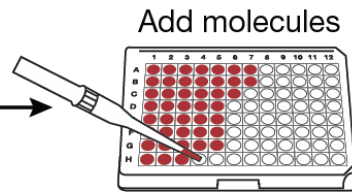
Fast implementation in QC lot release

Plate Ready-to-Assay Cells



0-4 h

Treat with Agonist/Molecule



16 h

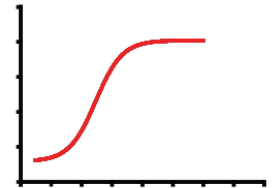
Add Detection Reagent 1

15 min

Add Detection Reagent 2

60 min

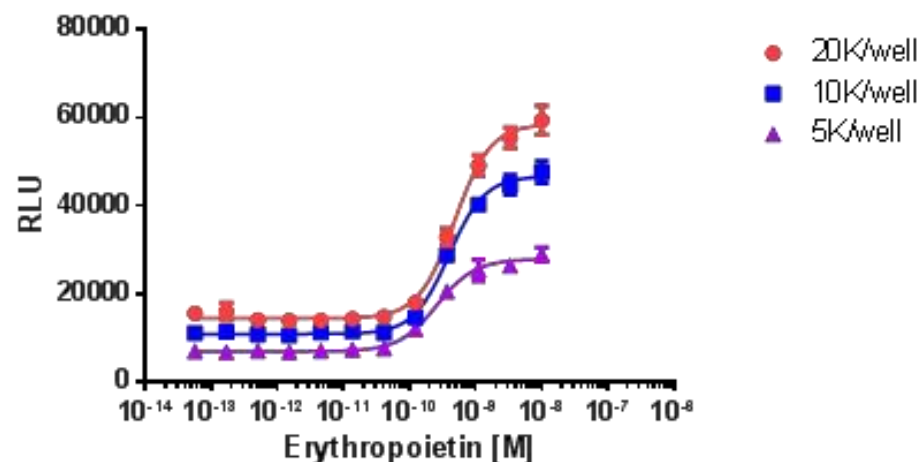
Read Luminescence



< 1 day

Assay Optimization: Cell Number & Plating Time

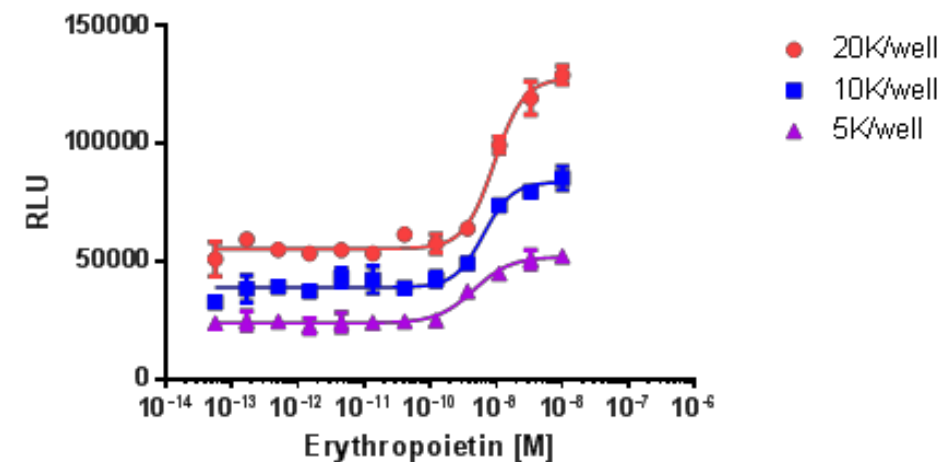
Plating Time: 4 hours



	20K/well	10K/well	5K/well
Sigmoidal dose-response (variable slope)			
Best-fit values			
Bottom	14493	10921	6976
Top	58805	46741	27890
LogEC50	-9.320	-9.403	-9.589
HillSlope	1.588	1.610	1.516
EC50	4.790e-010	3.955e-010	2.576e-010

S/B= 3.8 4.3 4.1

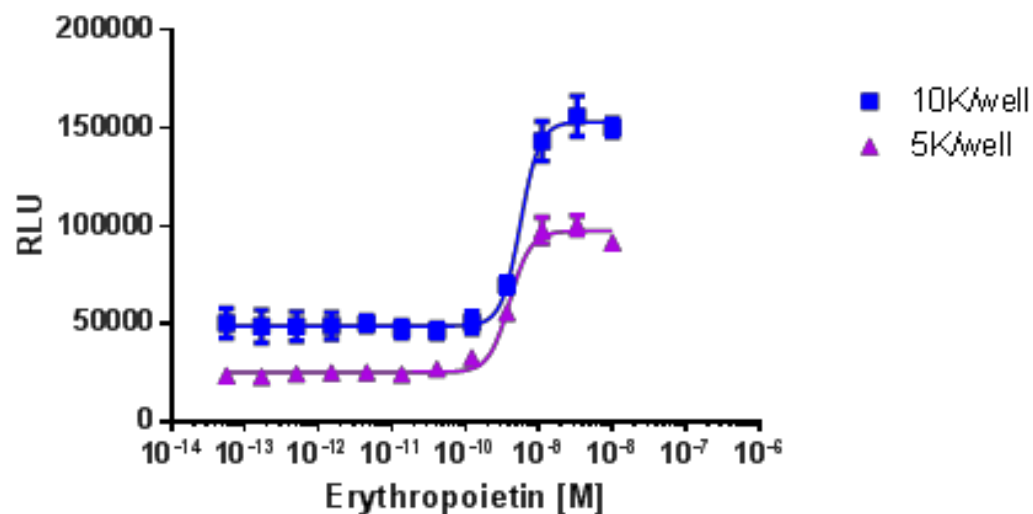
Plating Time: 24 hours



	20K/well	10K/well	5K/well
Sigmoidal dose-response (variable slope)			
Best-fit values			
Bottom	55486	39042	23906
Top	127989	83822	52046
LogEC50	-9.031	-9.193	-9.339
HillSlope	1.928	1.998	1.557
EC50	9.308e-010	6.405e-010	4.579e-010

S/B= 2.5 2.6 2.2

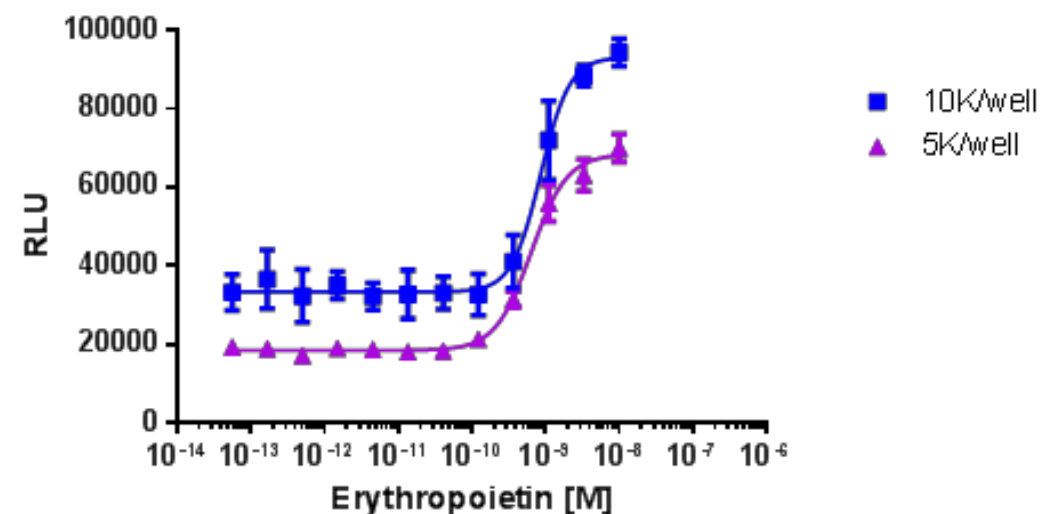
Plating Reagent: CP0



	10K/well	5K/well
Sigmoidal dose-response (variable slope)		
Best-fit values		
Bottom	48889	25327
Top	153173	97420
LogEC50	-9.251	-9.398
HillSlope	3.329	3.071
EC50	5.614e-010	4.016e-010

S/B= 3.0 3.9

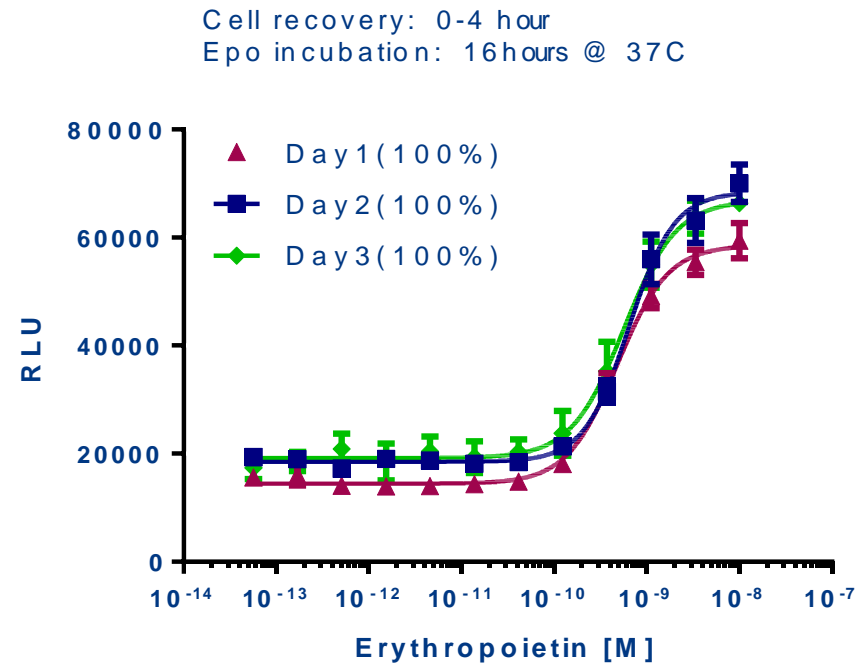
Plating Reagent: CP5



	10K/well	5K/well
Sigmoidal dose-response (variable slope)		
Best-fit values		
Bottom	33429	18518
Top	93135	68454
LogEC50	-9.064	-9.193
HillSlope	2.211	1.783
EC50	8.626e-010	6.412e-010

S/B= 2.8 3.6

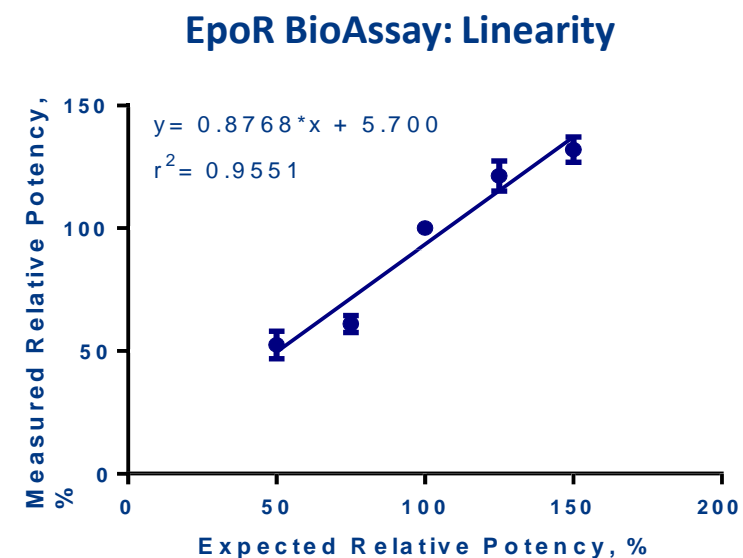
High Reproducibility of EPO assay



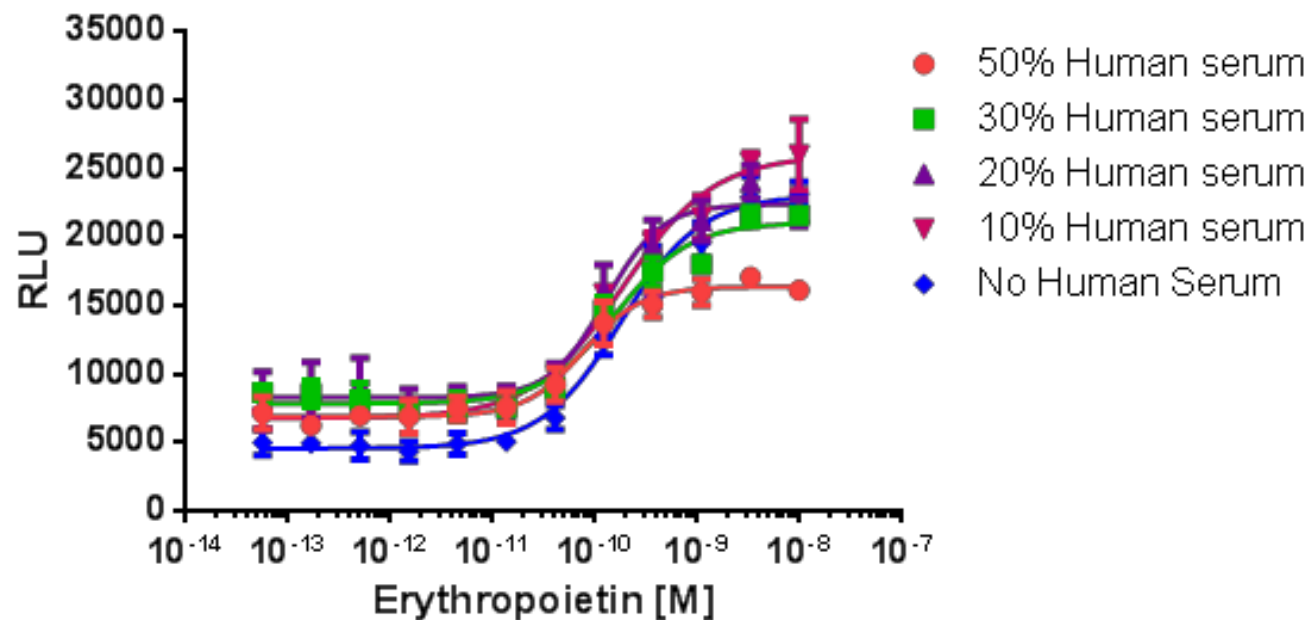
	Day 1 (100%)	Day 2 (100%)	Day 3 (100%)
HillSlope	1.586	1.783	1.530
EC50	4.790e-010	6.412e-010	5.601e-010
S/B=	3.8	3.6	3.2

Potency, Linearity, Precision & Accuracy

	Expected Potency	Measured Potency	Mean Potency	SD	Recovery	RSD, %
Day 1	150%	126	132	5.2	88	3.94
Day 2		135				
Day 3		135				
Day 1	125%	128	121.3	6.1	97.1	5.04
Day 2		116				
Day 3		120				
Day 1	75%	57	61	3.6	81.3	5.83
Day 2		62.2				
Day 3		63.8				
Day 1	50%	54.9	52.6	5.6	105.1	10.61
Day 2		46.2				
Day 3		56.6				



Precision: 6.36%
Accuracy: 92.9%



	HillSlope	EC50	S/B
50% Human serum	1.523	7.653e-011	2.3
30% Human serum	1.211	1.623e-010	2.5
20% Human serum	1.609	1.272e-010	2.5
10% Human serum	0.9753	1.886e-010	3.9
No Human Serum	1.110	1.803e-010	4.7

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:

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