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PathHunter® Growth Hormone Bioassay Kit

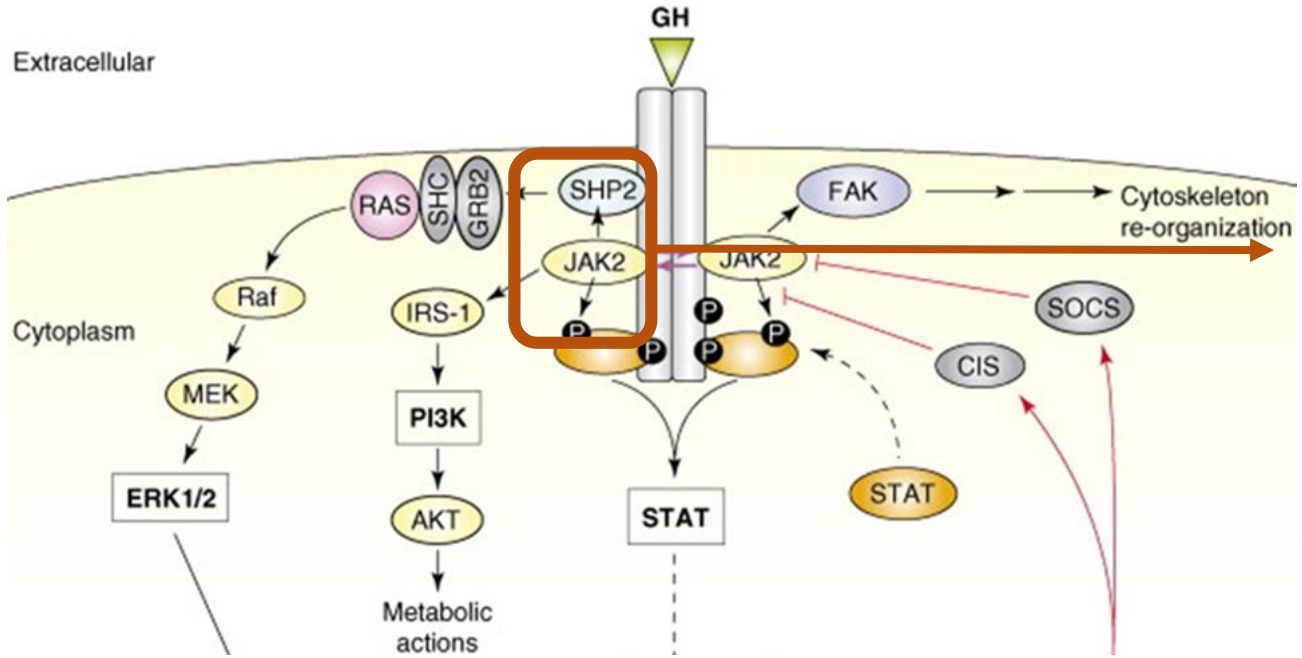
Qualified with Human Growth Hormone

93-0756Y3-00023 (2-Plate Kit)
93-0756Y3-00024 (10-Plate Kit)

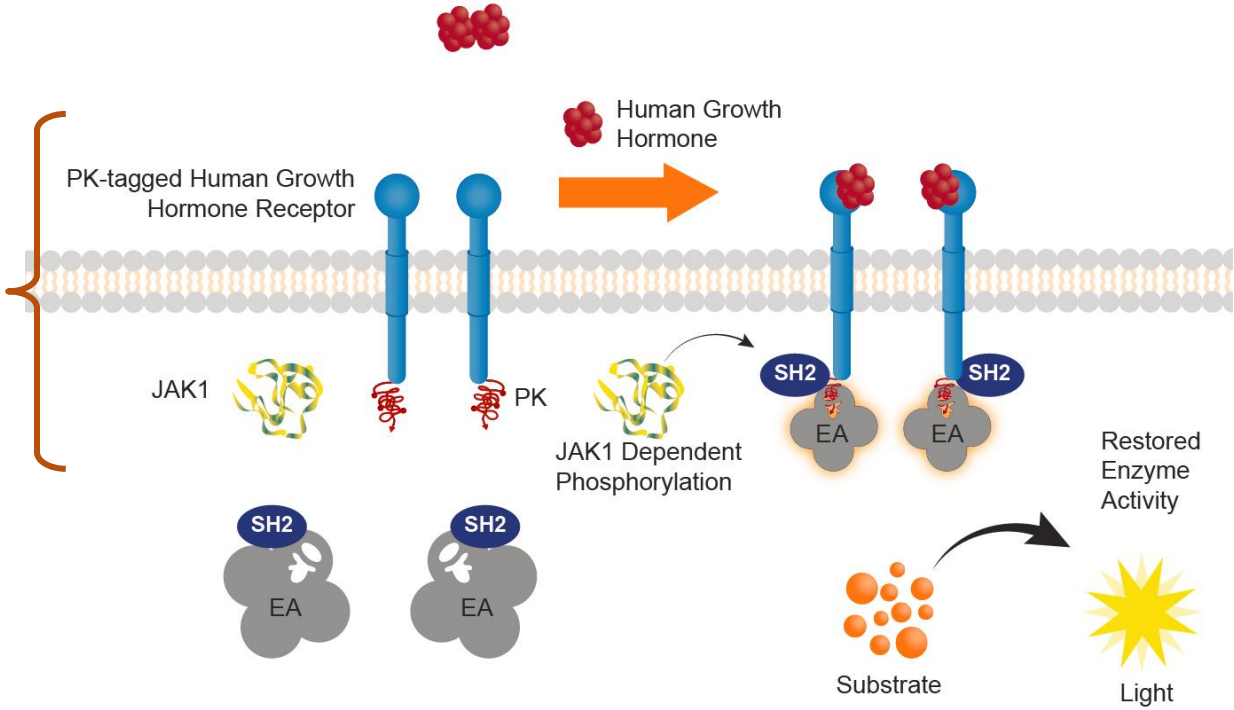
Growth Hormone Bioassay Principle

For treatment of growth hormone deficiency

Molecular Mechanism of Action



Assay Principle



Source: Rosenfeld, R.G., et. al. Trends in Endocrinology & Metabolism Volume 18, ISSUE 4, P134-141, May 01, 2007

PathHunter® Growth Hormone Bioassay Kit

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Kit Components

List of Components	93-0756Y3-00023	93-0756Y3-00024
PathHunter U2OS GHR-JAK1 Bioassay Cells	2 vials	10 vials
PathHunter Bioassay Detection Kit Detection Reagent 1 (mL) Detection Reagent 2 (mL)	 2 8	 10 40
AssayComplete™ Cell Plating Reagent 4	1 X 100 mL	3 X 100 mL
Assay Complete Dilution Buffer B5	1 X 100 mL	1 X 100 mL
Control Agonist (hGH)	1 vial	1 vial
96-well Clear-Bottom TC Treated, Sterile Plates w/lid	2 plates	10 plates

*Excess reagent may be left in the bottle, after using the volumes needed to run all the plates

Sample data

Growth Hormone 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} across entire plate
- Plate-to-Plate variability: 3 plates with full plate DRCs run on 3 days
- Slope consistency
- Accuracy, precision, linearity and parallelism of relative potency assay across range of 50-150% from two operators :
  - Assay developer
  - Assay qualifier

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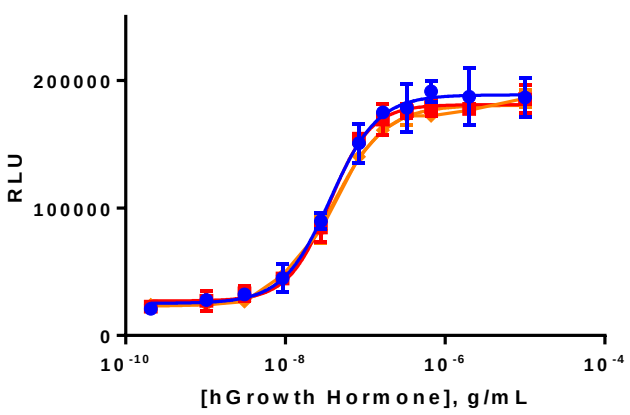
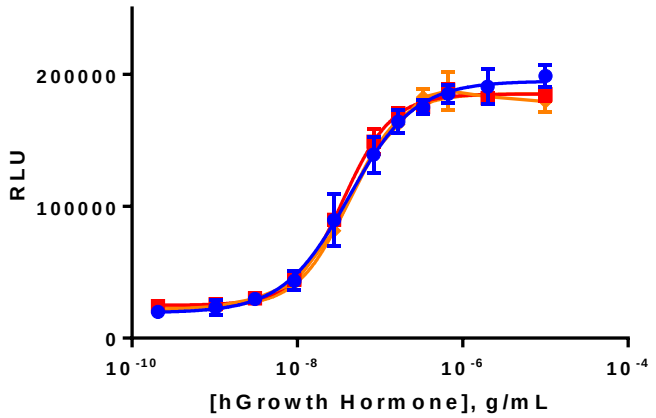
Plate to plate variability: 3 plates with full plate DRC - same day (Day 3)

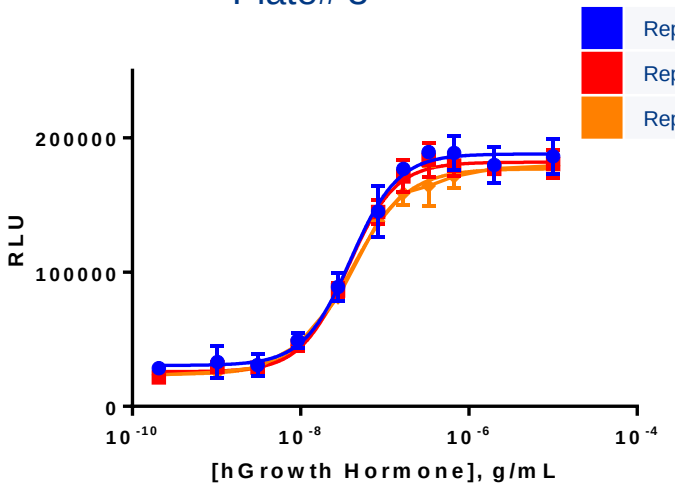
Plate# 1



Plate# 2



Plate# 3



Parameter	R1	R2	R3
S/B	7.9	7.8	7.8
Hill Slope	1.460	1.671	1.304
EC_{50} (ng/mL)	36.59	35.81	38.44

Parameter	R1	R2	R3
S/B	7.8	7.1	7.4
Hill Slope	1.093	1.462	1.411
EC_{50} (ng/mL)	42.08	36.03	41.90

Parameter	R1	R2	R3
S/B	6.6	7.6	8.1
Hill Slope	1.545	1.551	1.310
EC_{50} (ng/mL)	39.32	36.60	38.99

Growth Hormone Bioassay Qualification with hGH

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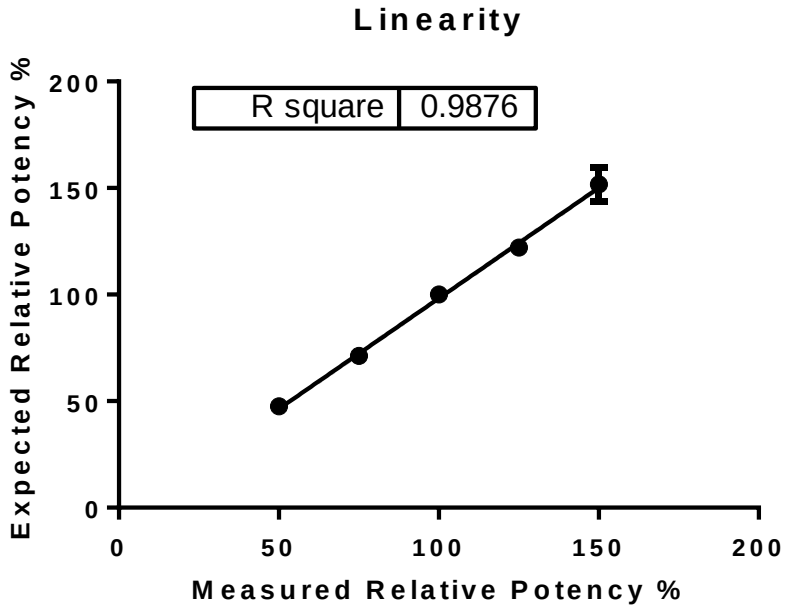
Relative Potency, Linearity, Accuracy and Precision

Expected RP (%)	Experiment #	Analyst #	Measured RP (%)	Average RP (%)	% RSD	% Recovery
150	1	1	154.2	151.8	5.2	101.2
	2	1	146.3			
	3	1	144.9			
	4	2	161.9			
125	1	1	126.2	122.1	3.2	97.7
	2	1	122.3			
	3	1	116.8			
	4	2	123.1			
75	1	1	71.2	71.3	4.3	95.1
	2	1	73.3			
	3	1	73.6			
	4	2	67			
50	1	1	45.9	47.6	7.4	95.2
	2	1	46.9			
	3	1	44.8			
	4	2	52.7			



Accuracy: 97.3%
Precision: 7.4%

Growth Hormone Bioassay Qualification with hGH

Assay Robustness

Repeatability and Intermediate Precision (Inter-Plate)

Plate #	Experiment #	R2	S/B	EC_{50} (ng/mL)	Mean EC_{50} (ng/mL)	SD	%RSD
1	R1	0.984	7.9	36.59	38.42	2.39	6.2
	R2	0.992	7.8	35.81			
	R3	0.995	7.8	38.44			
2	R1	0.989	7.8	42.08			
	R2	0.998	7.1	36.03			
	R3	0.994	7.4	41.90			
3	R1	0.9843	6.6	39.32			
	R2	0.9915	7.6	36.60			
	R3	0.9943	8.1	38.99			

Intermediate Precision (Inter-Day)

Day	EC_{50}, ng/mL	Mean EC_{50}, ng/mL		%RSD, EC_{50}
1	52.1	47.33	7.71	16.35
2	51.5			
3	38.4			

Intermediate Precision (Inter-Plate): 6.2%
Intermediate Precision (Inter-Day): 16.4%

Growth Hormone Bioassay Qualification with hGH

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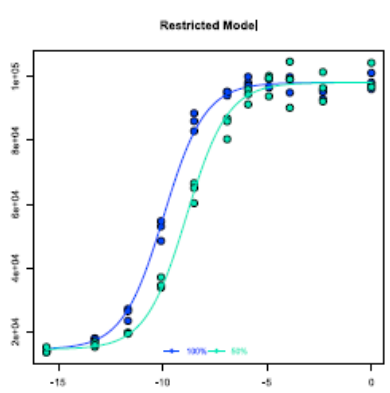
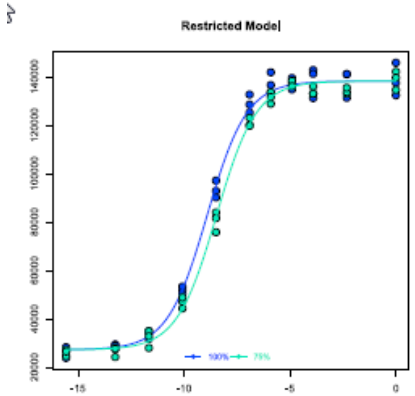
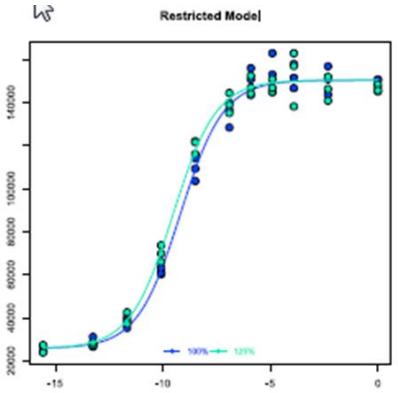
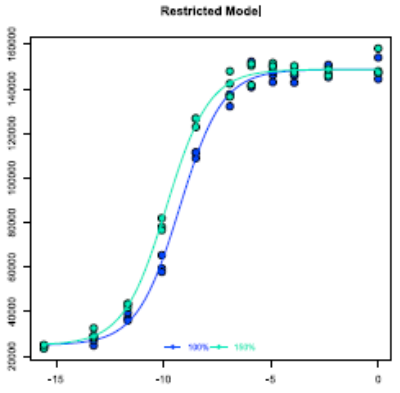
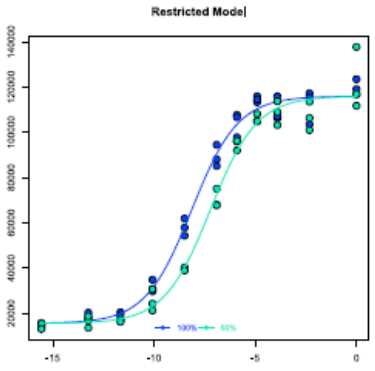
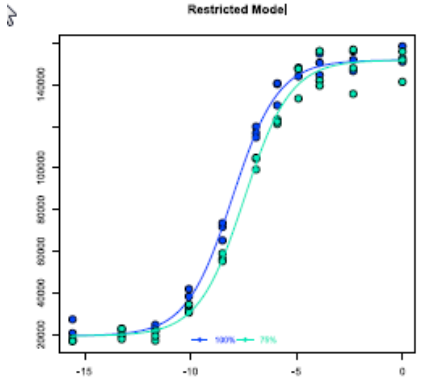
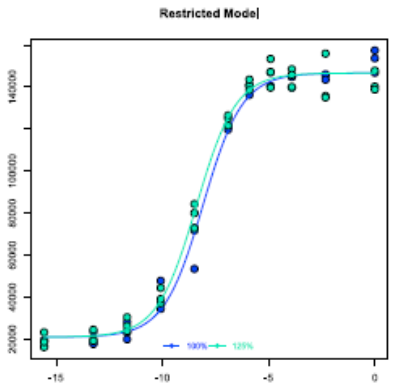
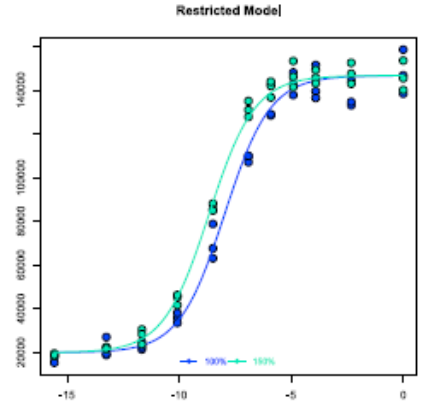
Parallelism and Potency Estimation (PLA):

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

Reference Sample

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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