Bioassay
Bioanalytical Contract Laboratory
A Reaction Biology Company

Erythropoietin (EPO)

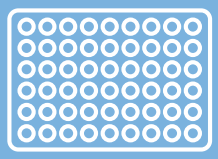
Luminescence Cell-Based Potency Assay for Erythropoietin

Cell-based potency determination using BAF3 cells with overexpressed huEpoR receptors. ICH Q2(R2)-qualified methodology measuring ATP production after receptor activation. Suitable as 3R-compliant alternative for batch release and stability evaluation under GMP conditions.

WHO Standard-Based Methodology

- **Reference Standard:** WHO Standard Erythropoietin (NIBSC Code: 11/170)
- **Alternative to:** Ph. Eur. monograph 1316 Erythropoietin concentrated solution (ECS)

Ahead of Regulatory Trends



ICH Q2(R2)-qualified methodology supports your workflows for emerging acceptance of in vitro alternatives.

Screen Smarter, Save Faster

Eliminate upfront animal testing costs for candidate screening and early validation, saving upfront on in vivo models.

WHO Standard Foundation

7.16% mean GCV precision using NIBSC 11/170 reference standard accelerates your path to in vivo replacement or biosimilar submissions.

Technical Specifications

- Luminescence detection (RLU)
- 10 dose steps, triplicates per step
- 1 test sample + assay control
- 4-PL fit for potency determination
- USP <1032> equivalence margins

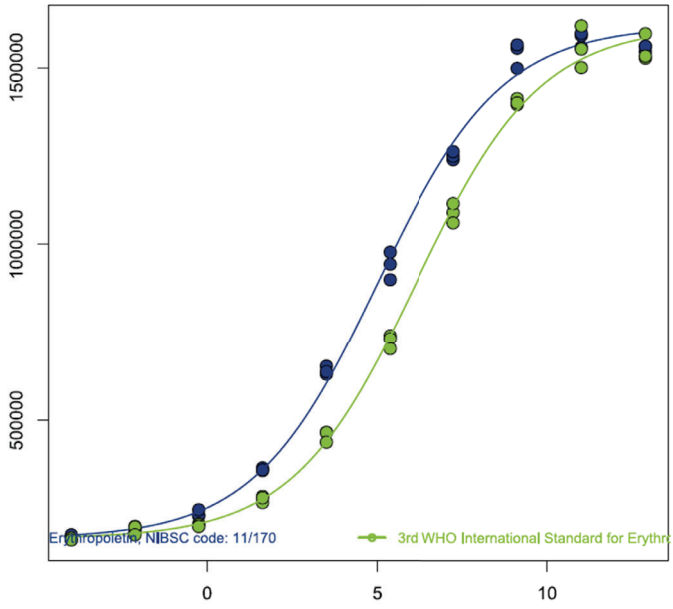
Applications

- Biosimilar characterization
- Batch release and stability testing
- Method qualification per ICH Q2(R2)
- Regulatory submission support
- Clinical trial material testing

Potency Assay Example

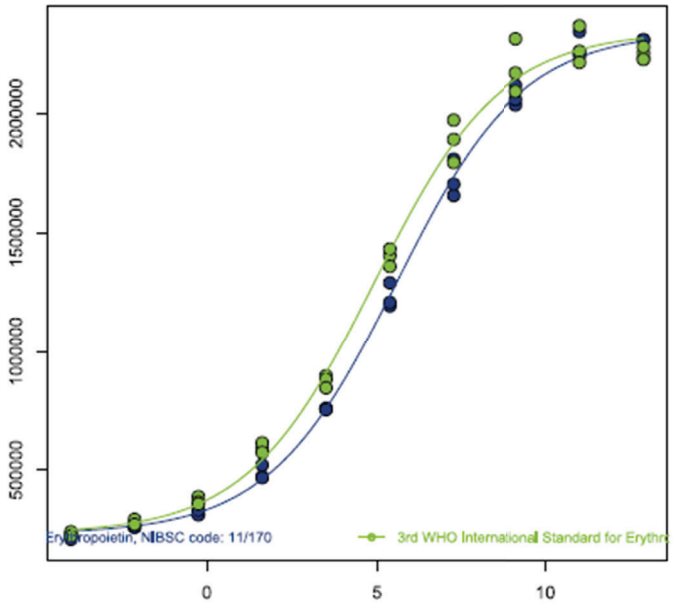
Assay Setup and Evaluation

- WHO Standard Erythropoietin (NIBSC Code: 11/170) was used with 50% and 150% potency level as sample
- A 4-PL fit was used for potency determination
- Titration was performed over 10 dose steps in triplicate
- Assays “passed” according to Equivalence Margins (USP <1032>)
- Relative Light Units (RLU) are depicted on graph as response
- Erythropoetin concentration depicted on graph as relative dose (log base 2)



Potency Estimation 50% vs. 100%

Estimated vs Stated Potency	51.7% vs. 50.0% (weighted mean)
95.0% Confidence Interval	0.48312 – 0.55321 (weighted mean)
Relative Confidence Interval	93.45% – 107.00% (13.55%)



Potency Estimation 150% vs. 100%

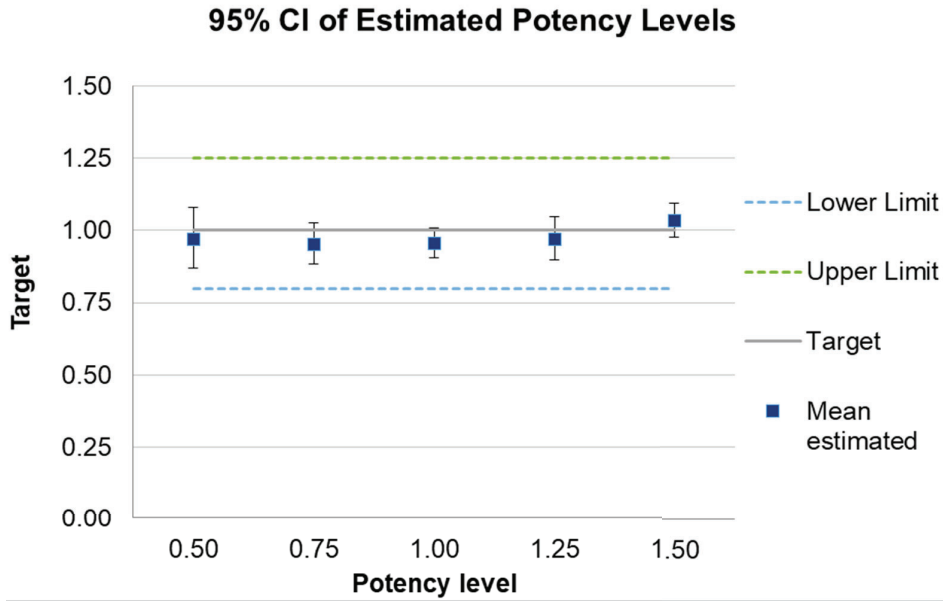
Estimated vs Stated Potency	150.9% vs. 150.0% (weighted mean)
95.0% Confidence Interval	1.39689 – 1.63071 (weighted mean)
Relative Confidence Interval	92.55% – 108.05% (15.50%)

Method Qualification

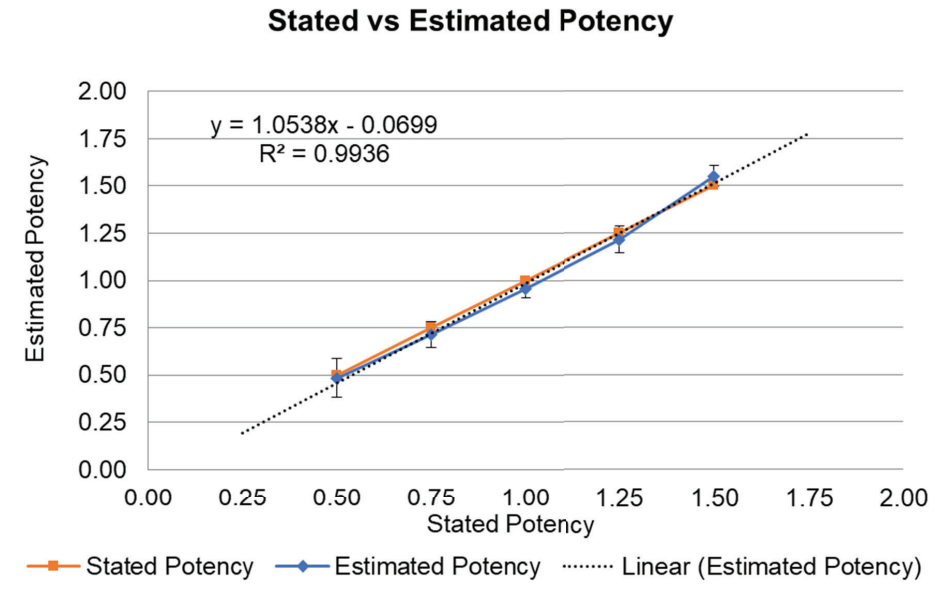
Result Summary

- Five potency level investigated (n = 6 each)
- Assessment of accuracy, linearity, precision (overall and intermediate), and repeatability

Accuracy



Linearity



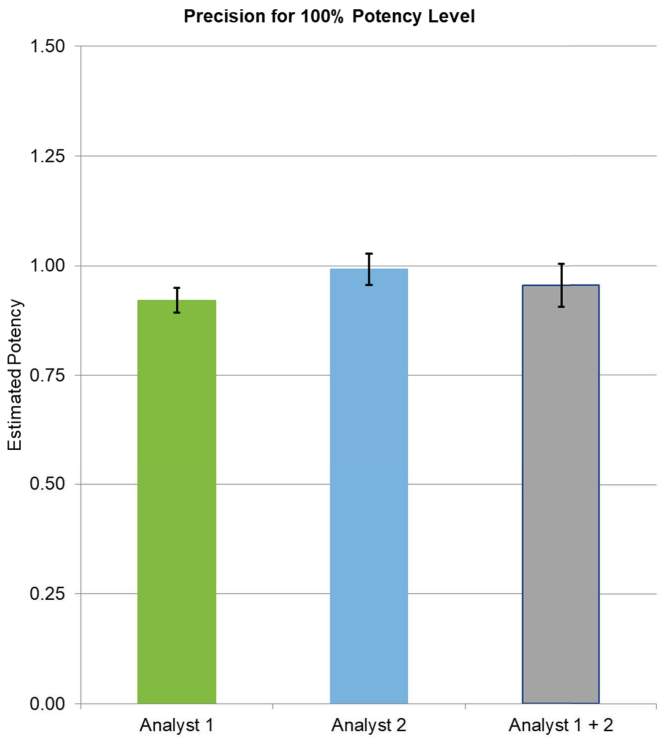
Precision (Overall)

RP Level	Geometric Mean	%GCV
0.50000	0.48453	10.63
0.75000	0.71390	7.05
1.00000	0.95539	5.08
1.25000	1.21361	7.38
1.50000	1.55188	5.69
	Mean	7.16

Precision (Intermediate)

Mean	0.96624	**GM**	0.96431
SD	0.068	**GSD**	0.069
CV%	7.04	**GCV%**	7.13

Repeatability (n = 6, Analyst 1)



Let's Discover Together.

Position your EPO program ahead of the curve. Speak with an expert.

Version 1.2
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