

# Bioassays: Industry Perspective (USA)

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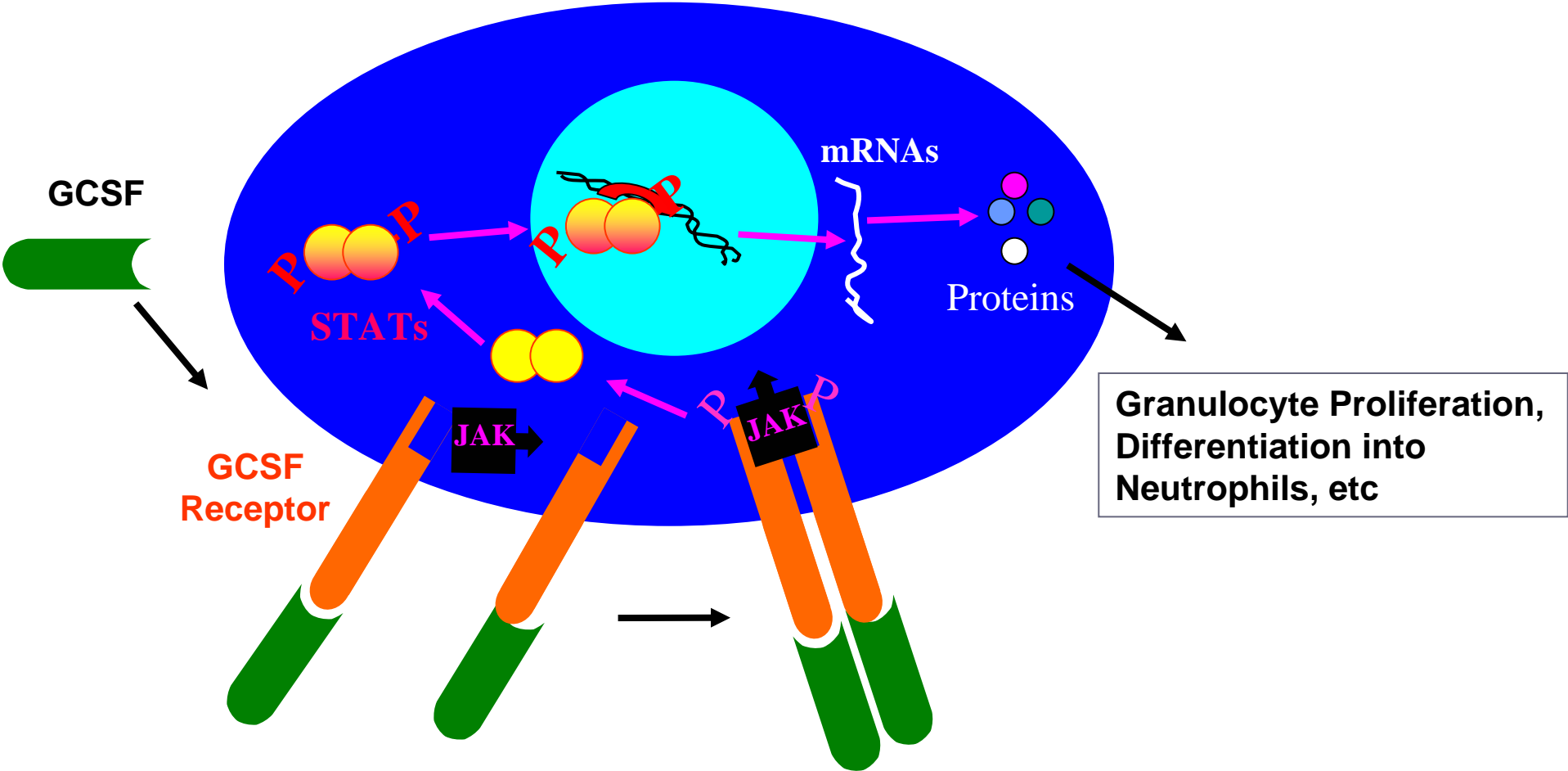
USP India ASM 2008, Hyderabad February 6, 2008

# Potency Bioassays

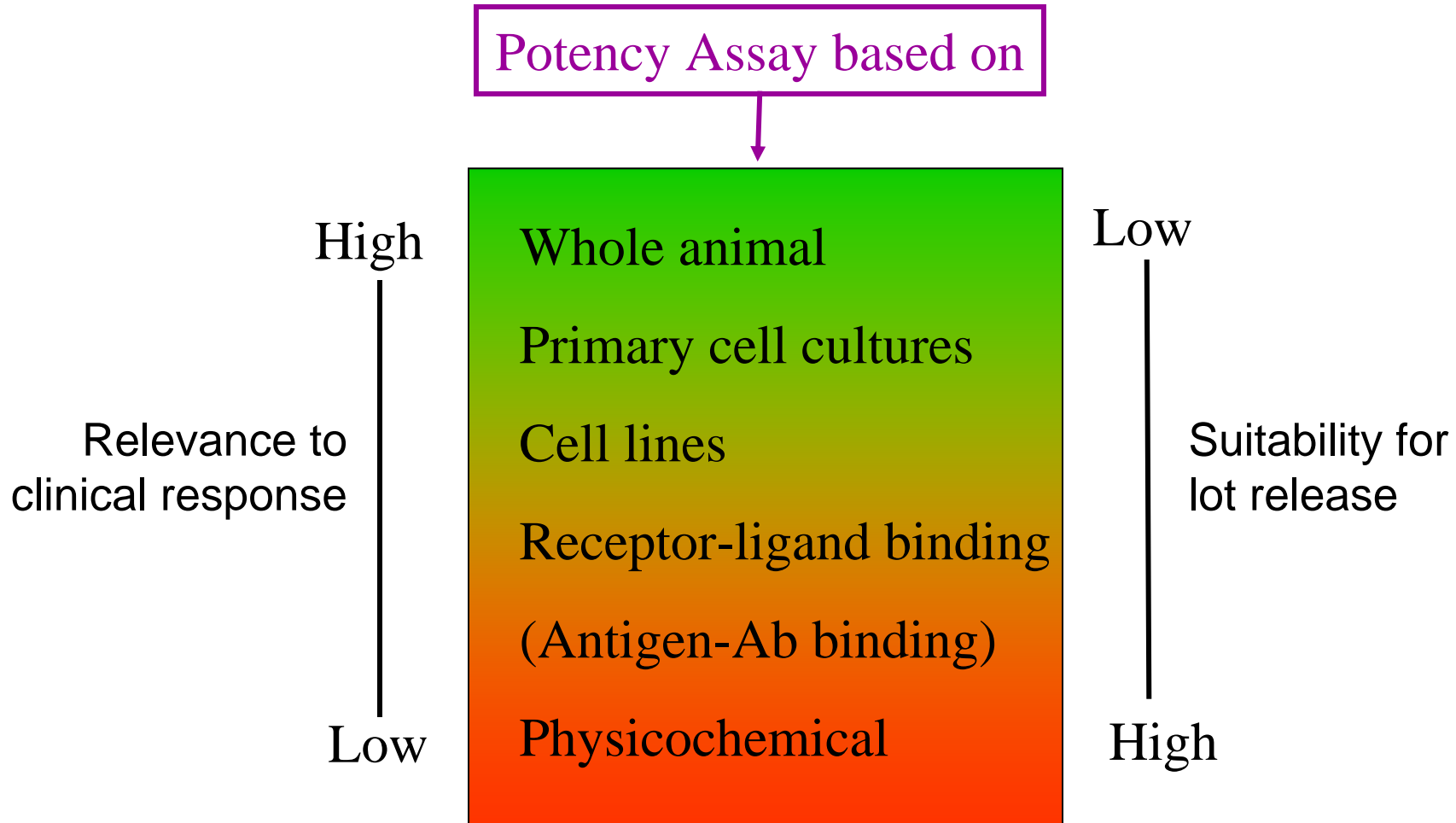
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- Potency is a quantitative measure of biological activity
- Potency is measured using a bioassay (also referred to as biological activity assay)
- Potency assays used for lot release and stability of commercial product have to meet stringent accuracy and precision criteria
- In the eyes of the Regulatory Agencies, potency is the single most quality attribute of a biologic product

# GCSF Cellular Mechanism of Action



# In Vivo and In Vitro Potency Assays



# Evolution of Bioassay for GCSF

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Cell proliferation assay using murine cell line NFS-60 and  $^3\text{H-TdR}$

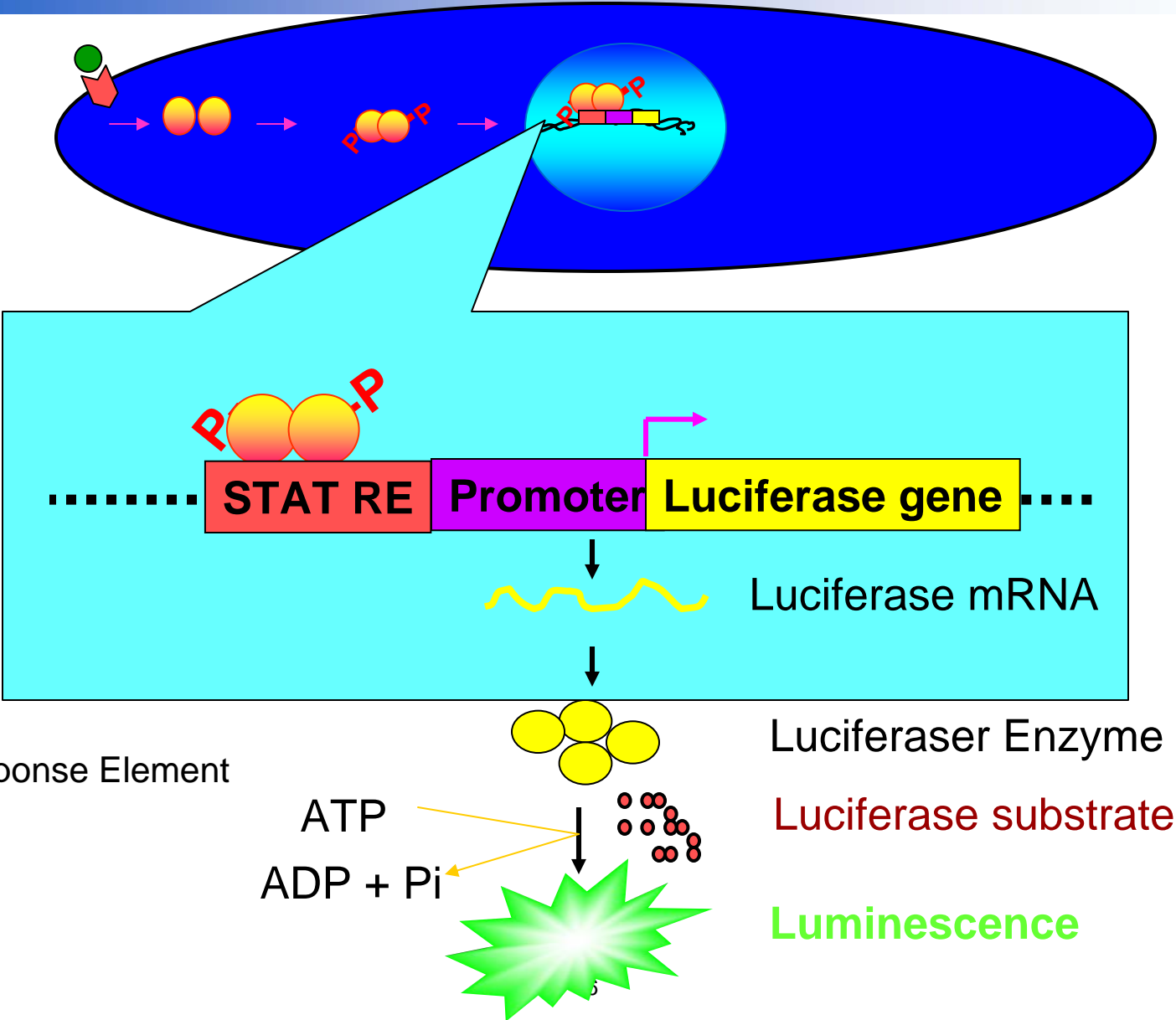


Cell proliferation assay using murine 32D clone3  
grown in GCSF & AlamarBlue readout



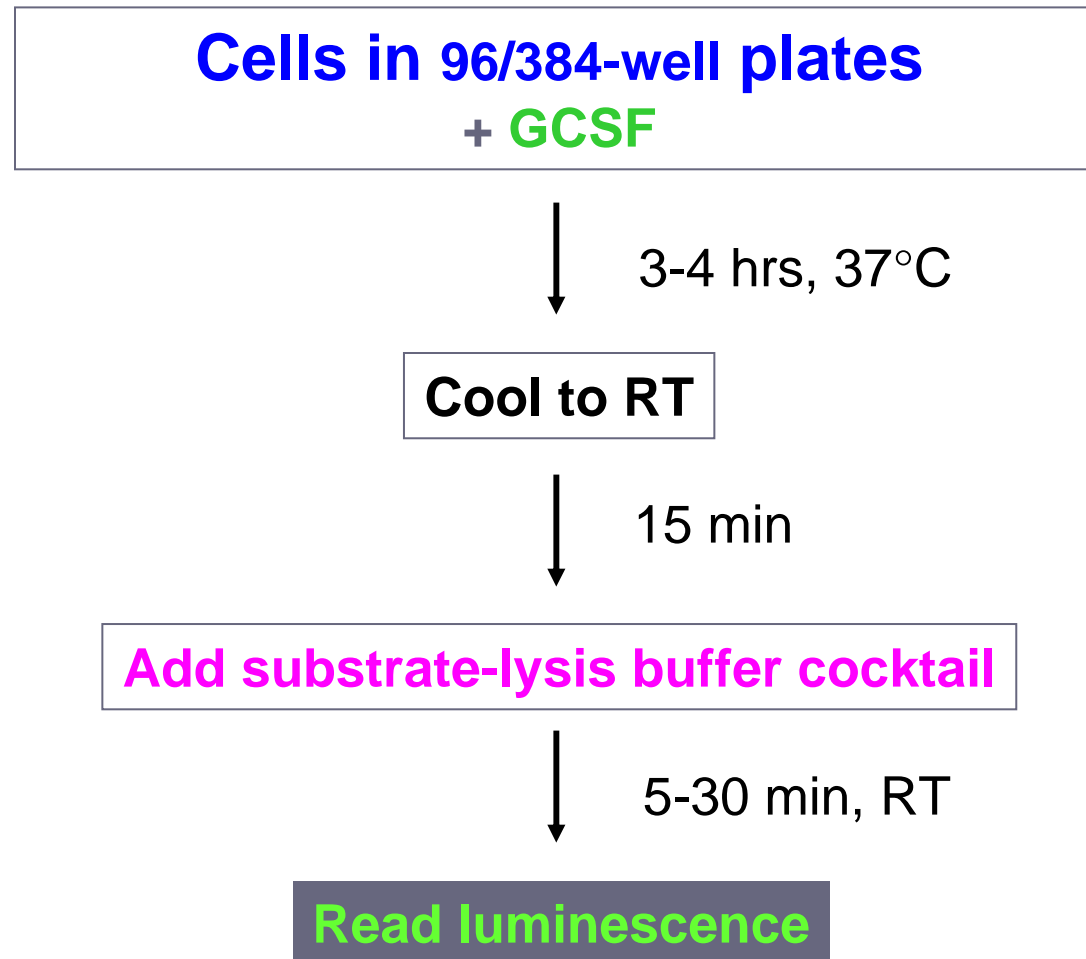
Rapid assay based on reporter gene expression  
using 32D clone transfected with huGCSFR  
and luciferase reporter gene

# Principle of Reporter Gene Assay



# Simplicity of Reporter Gene Assay

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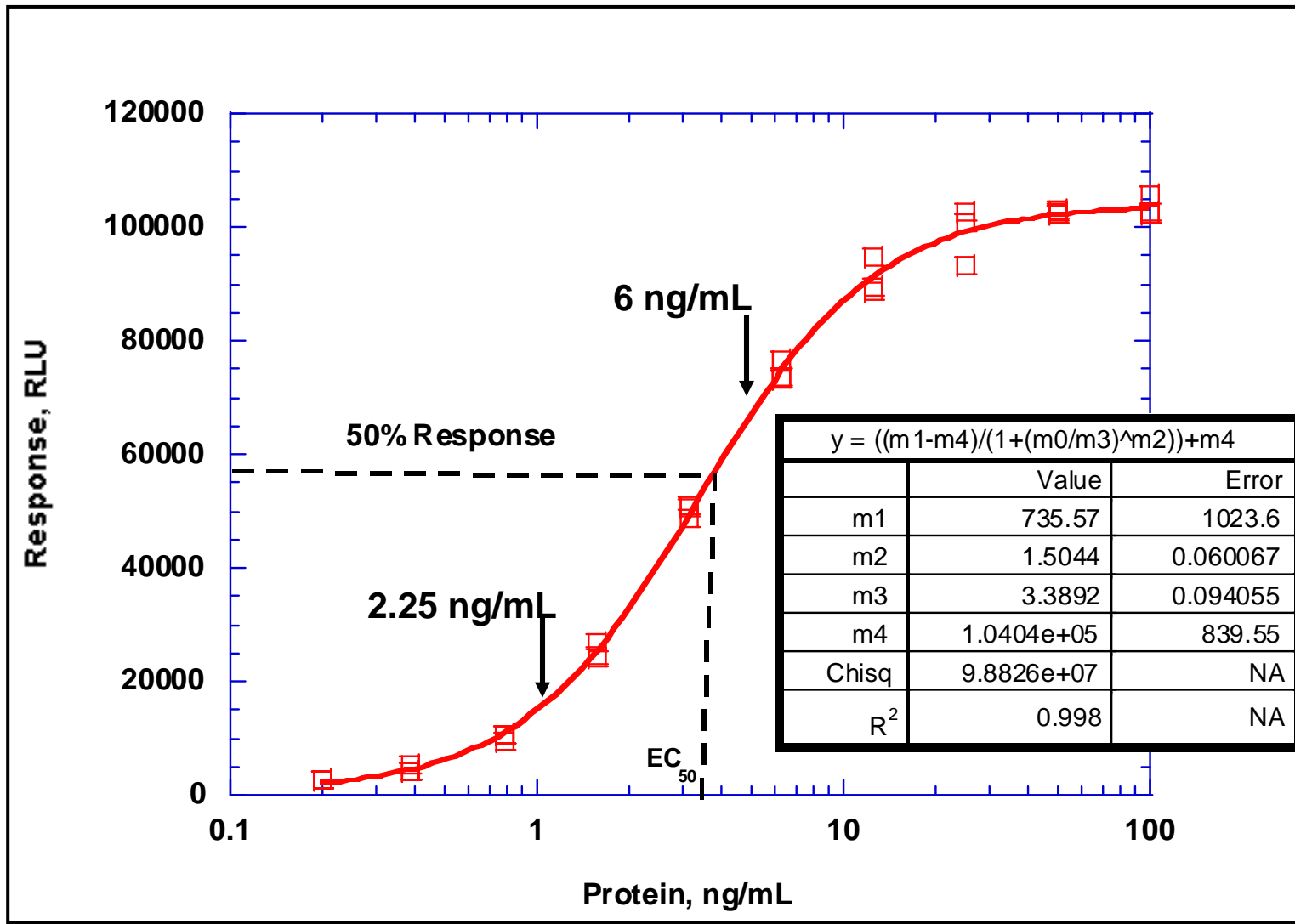


# Advantages of Reporter Gene Assay

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- Shorter duration ~4 hours
- Sterility not necessary
- Less susceptible to temperature and CO<sub>2</sub> gradients during incubation
- Data available more rapidly for lot release
- Homogeneous assay format
- Higher throughput
- Higher precision
- Troubleshooting is faster

# Reporter Gene Assay Dose Response Curve



# Data Analysis

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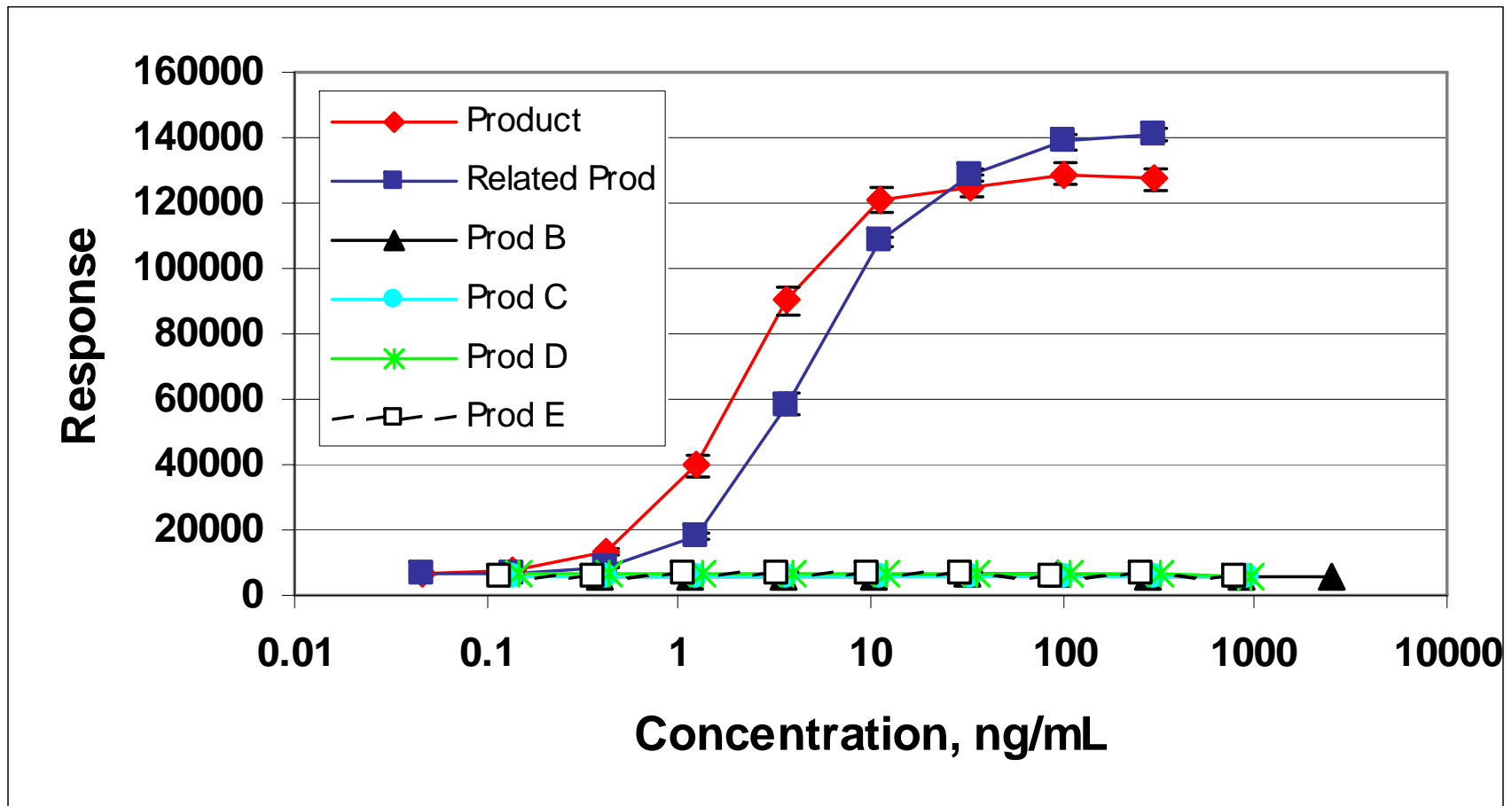
- Five doses in the linear range are tested
- Minimum 3 consecutive doses for linear regression and parallelism assessment
- Parallelism based on current USP and EP monographs using p value approach
  - Will be replaced with equivalence approach (USP<111>)
- Reportable value is a weighted mean from three independent determinations

# Summary of Validation Results

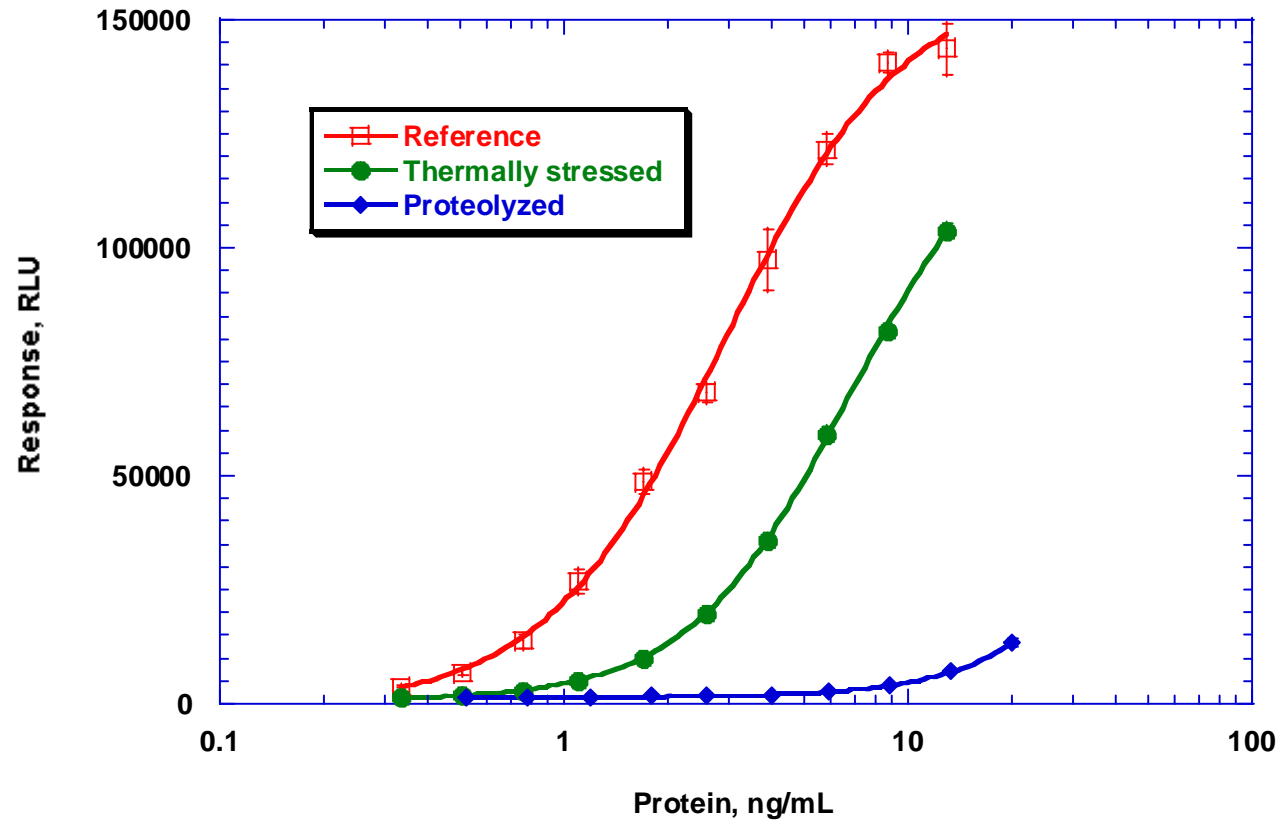
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- Repeatability CV <4%
- Intermediate precision CV <7%
- Accuracy: Spike recovery for 70%,-130% potency samples is greater than 90%
- Specificity: Placebo has no activity even at 100 times concentration
- Additional experiments
  - Specificity
  - Stability indicating property
  - Location effects
  - Robustness

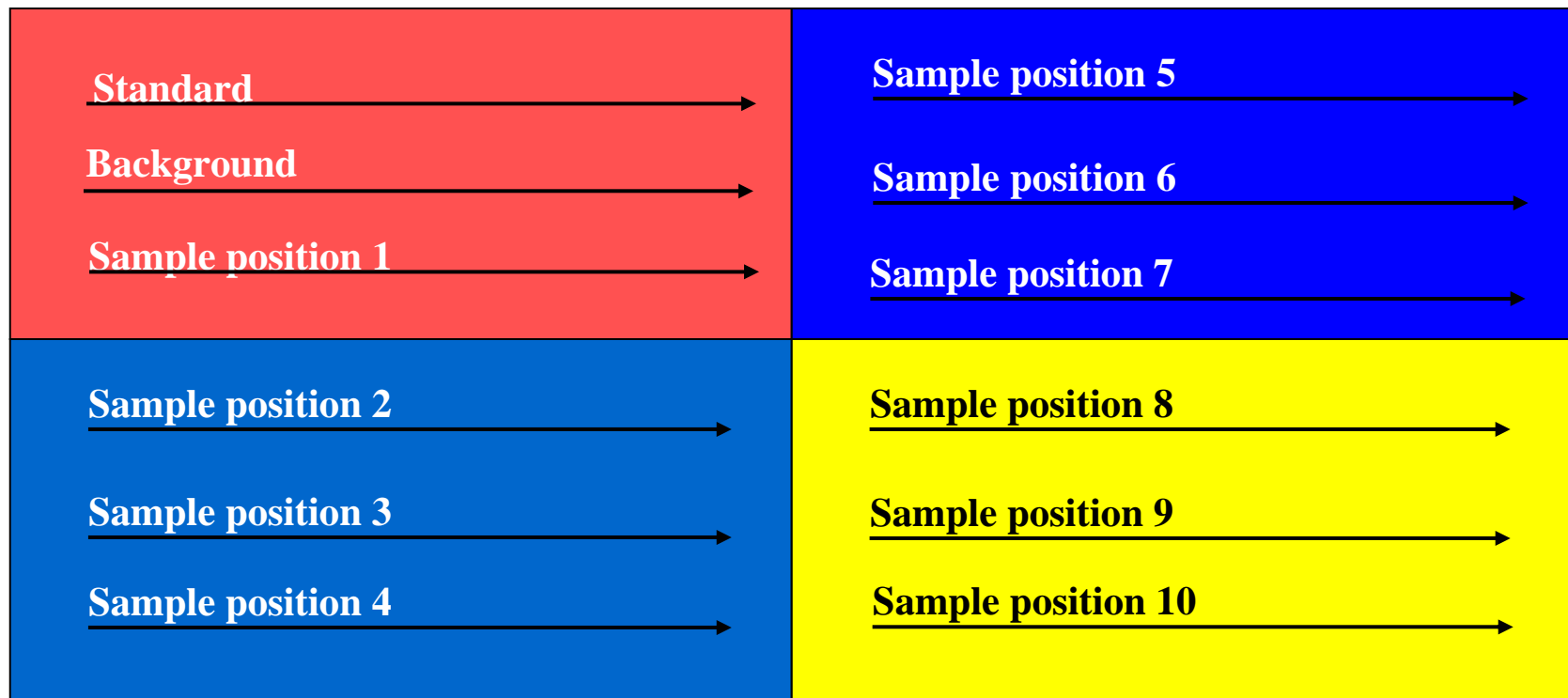
# Specificity of Reporter Gene Assay



# Activity of Stressed sample in Reporter Gene Assay



# Plate Design for Randomization of Sample Position



**5 concentrations, high to low**

# Plate Design for randomization between plates

I RED	III BLUE
II GREEN	IV YELLOW

I BLUE	III RED
II YELLOW	IV GREEN

**Plate 2**

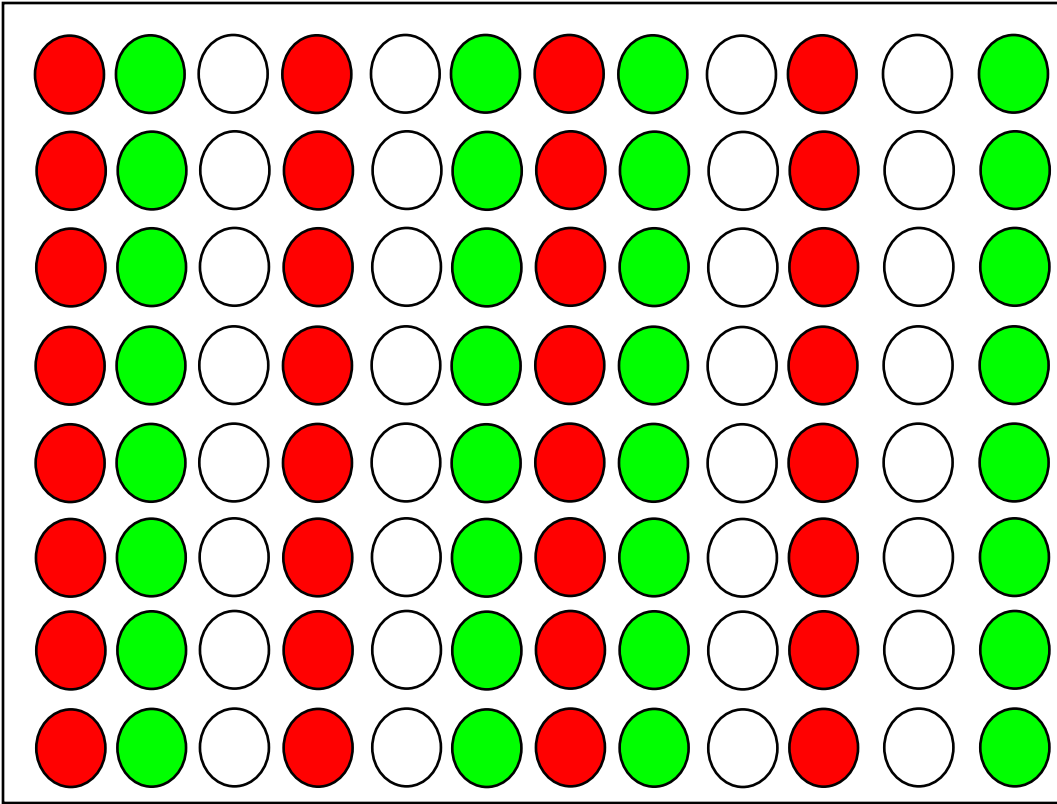
I YELLOW	III GREEN
II RED	IV BLUE

**Plate 4**

I GREEN	III YELLOW
II BLUE	IV RED

# Minimize Location Effects with Strip Plot Designs &/or Semi-randomization

● Reference      ● Test Sample #1      ○ Test Sample #2



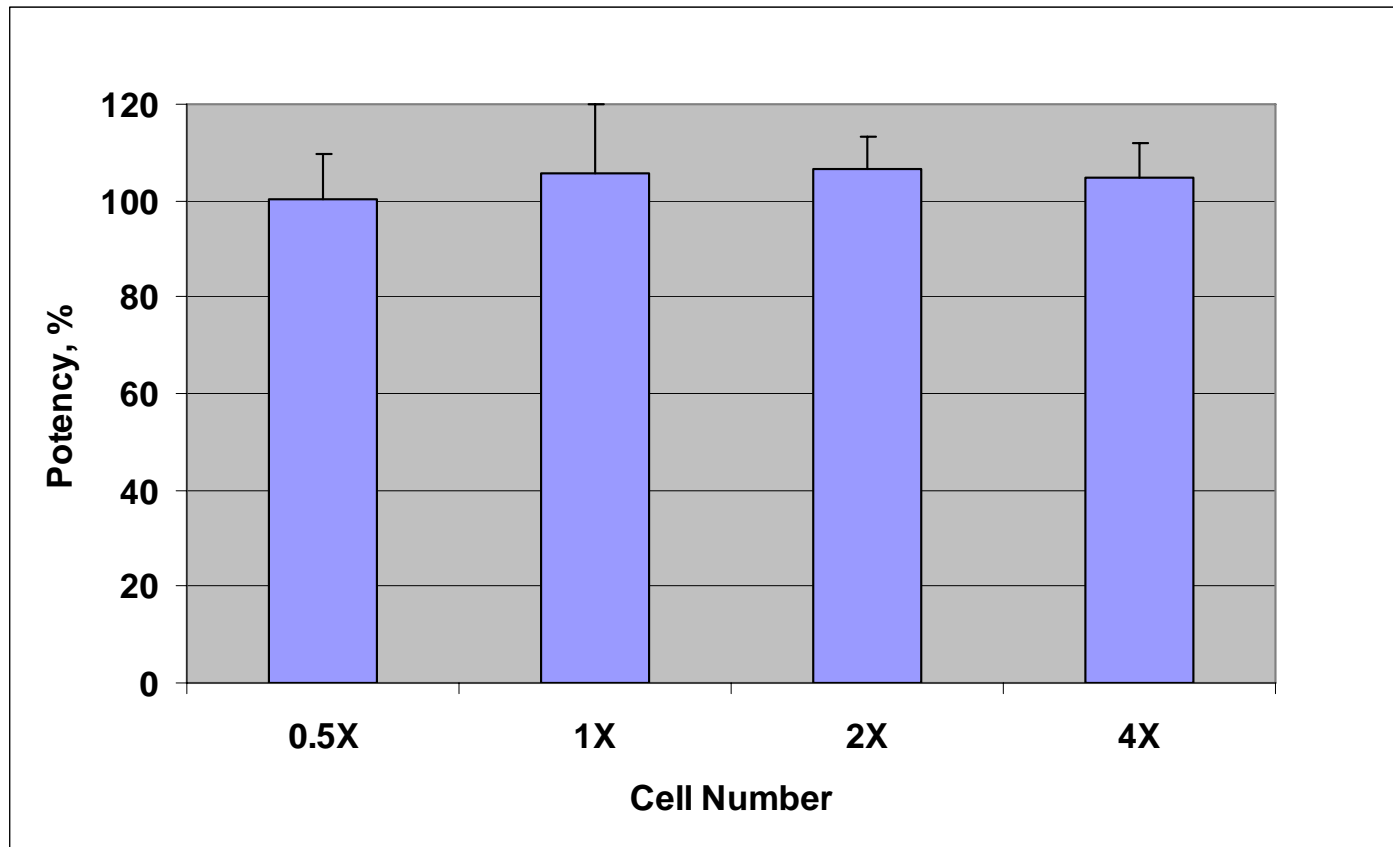
# Robustness

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- Robustness not usually included in validation report
- Identify critical factors in the bioassay
- Use appropriate DOE
- +, - Boundaries should be chosen based on expected variability under operating conditions

# Robustness: Cell number per well

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# Example of Robustness DOE

Experiment (run)	Cells per well (0 = 10000, - = 7500, + = 12500)	First incubation time (0 = 24 hrs, - = 22 hrs, + = 26 hrs)	Second incubation time (0 = 24 hrs, - = 23 hrs, + = 25 hrs)
1	0	0	0
2	-	-	+
3	+	-	-
4	0	0	0
5	-	-	-
6	+	+	-
7	0	0	0
8	-	+	+
9	-	+	-
10	0	0	0
11	+	+	+
12	+	-	+

# Assigning Value to Reference Standard

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- Test for equivalency with international reference standard and then assign 100% or the same specific activity
- Alternatively, assign specific activity against the international standard
- Submit a clear protocol with the license for generating subsequent lots of internal standard
  - Characterization beyond lot release assays
  - Replication far more than lot release
  - Acceptance criteria tighter than lot release specifications

# Stability Monitoring of Reference Standard

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- Maintain a formal stability program for Ref. Standard
- Have clear acceptance criteria
- Check freeze/thaw stability
- Utilize stability indicating methods other than the potency assay
- Store at multiple locations
- Address out-of-specifications
- If exact international reference is not available, use a closely related international reference standard and monitor the relationship factor

# Acknowledgements

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- Jill Crouse, Ph.D., Principal scientist
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- GMP Bioassay Lab