Development & qualification of a potency assay: guidelines and strategy

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Poster 0068

Abstract

Gene therapy products hold immense promise for treating various genetic disorders; however, ensuring the potency, efficacy, and safety of such therapies necessitates the development and qualification of robust potency assays. This poster delves into the meticulous process of developing and qualifying a potency assay, with a focus on strategies and guidelines.

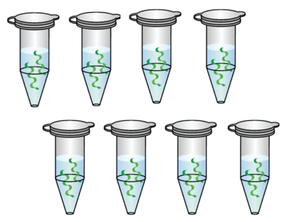
The development phase commenced with a thorough optimization of the workflow. Parameters critical to assay performance, including seeding density, transduction time, and the range of multiplicity of infection (MOI), were systematically varied and evaluated. This optimization aimed to enhance assay sensitivity and reliability while minimizing variability.

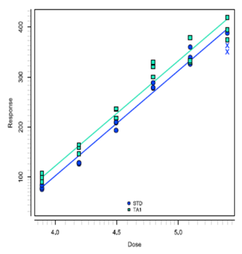
Analysis and qualification of the potency assay (measuring protein activity levels) involved a comprehensive assessment of pre-qualification and qualification data. Key performance parameters such as specificity, repeatability, intermediate precision, accuracy, linearity, and range of the test method were rigorously evaluated against predefined criteria outlined in the qualification protocol.

A critical aspect of the qualification process was the selection of appropriate reagents. Reagents play a pivotal role in assay performance and reliability. Careful consideration was given to the sourcing, characterization, and validation of reagents to ensure consistency and reproducibility across experiments.

Potency Assay Workflow[1]

Cell Model Infection & Protein Expression → Harvest and Lysis → Analytical Method (Specific to Product Function) → Data Analysis & Reporting

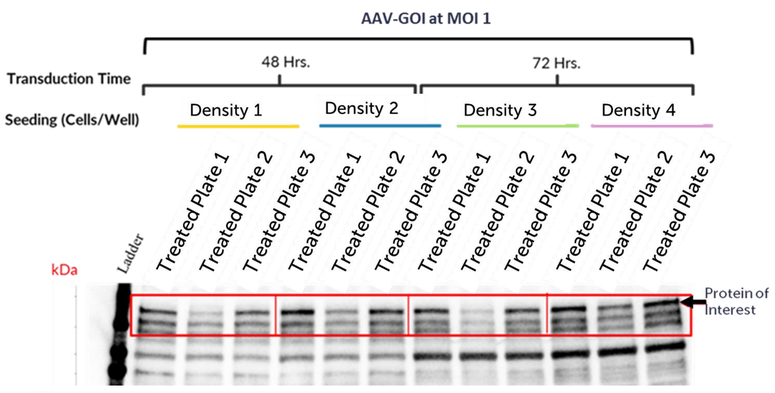




- Literature search and proof-of-concept studies.
- Assay development and parameter optimization.
- Assay qualification per ICH guidelines.
- Assay validation (future direction)

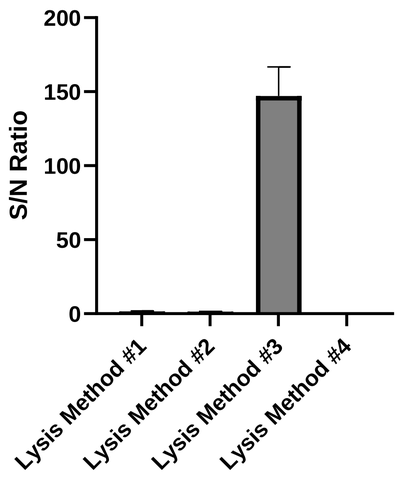
[1]This portrays the workflow of a potency assay (measuring activity) developed in house.

Optimization of Transduction Parameters



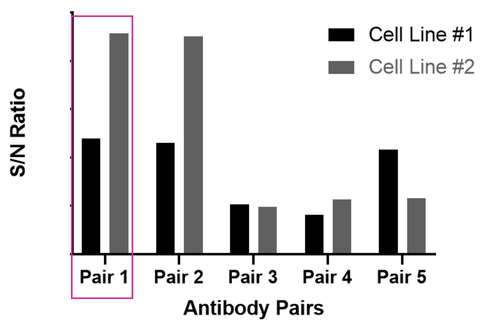
- The impact of various plate treatments, transduction times, and seeding densities were evaluated on protein expression levels using western blot analysis.
- Our results indicated that plates with treatment 1 and treatment 3 exhibited comparable levels of protein expression.
- Treatment plate 1 @ seeding density 4 was selected as most optimal.

Lysis Buffer Comparison



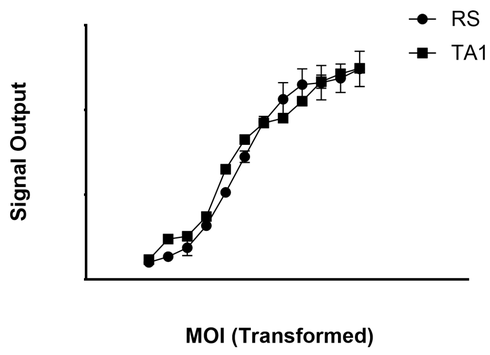
- The cell lysis method for protein harvest had a significant influence on the signal output for the potency assay.

Antibody Screening



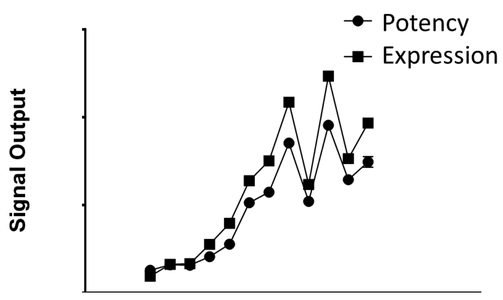
- Several antibodies, custom and commercial, were screened for final potency (activity levels) output assay.
- The pair with the greatest S/N ratio was considered the most optimal.

MOI Range Analysis



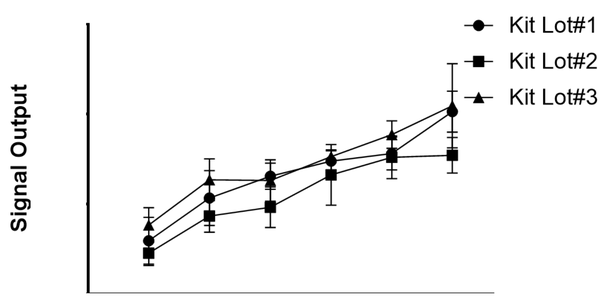
- MOI range of 12 consecutive points was assessed to determine optimal reliable range for reference and representative test article for use in a relative potency assay with parallel line analysis.

Potency (GOI activity) vs. Expression (Protein levels)



- Potency (activity) and expression (protein levels) were compared using the same samples and the same analytical output method.
- Similar signals and a trend was observed, showing a correlation between amount of protein and activity levels.

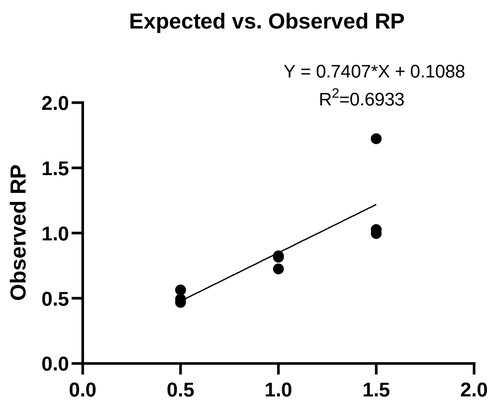
Custom Kit Bridging for GMP and non-GMP kits



- Three different lots of custom-made reagents for the potency assay (activity) were compared for bridging between the non-GMP and GMP lots.
- All lots showed similar signals and gave relative potency values (activity levels) as expected.

Assay Qualification

Parameter	Result
Accuracy	%Recovery 50% level= 94-113% 100% level= 73-83% 150% level= 67-115%
Linearity	R^2=0.69
Intermediate Precision	%GCV of RP (n=12) (2 analysts) at 100% of TA= 28%
Repeatability	%GCV of RP (n=6) (1 analyst) at 100% of TA= 18%
Specificity	Irrelevant AAV RP= 0% FFB RP= 0%
Range	RP= 50-150%



- Qualification parameters were tested following the ICH2 (R2) guidelines. Accuracy and precision met criterion for 100±35% recovery, and ≤30% CV, respectively.
- Range of assay was determined to be 50-150% and assay was specific.
- Analysis of all results were performed using PLA Stegmann analysis for relative potency.

Summary

The potency assay demonstrated robust performance across all critical parameters. Specificity assays confirmed the ability of the assay to accurately measure the potency without interference from other components. Repeatability and intermediate precision studies demonstrated the assays' reliability and consistency within and between experiments (%GCV≤30%) . Accuracy assessments confirmed the assays' ability to provide results close to the theoretical potency values (%recovery= ±35%). Linearity and range studies established the assay's ability to accurately measure potency over a broad range of concentrations (Range= 50-150%, R^2=0.69). This assay was determined to be fit-for-use in a Phase 2/3 program.

The development and qualification of a potency assay by employing meticulous cell line engineering strategies and adhering to guidelines represent a significant milestone in ensuring the efficacy and safety of this gene therapy products.

Aim higher

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