SARS CoV-2 Vaccines
Potency Assay Development

Potency Determination of mRNA Vaccines

- Flow cytometry based analytical method
- Measurement of antibody binding to spike protein after transfection
- Robust and precise assay, optimized by testing a wide variety of parameters
- Two assay formats available (2 sample, 10 dilution points; 4 samples + control sample, 8 dilution points)

- Suitable for batch release and stability evaluation under GMP conditions
- Time-efficient due to established 2 days protocol
- Adaptable to SARS CoV 2 variants

Procedure
### Principle of the Potency Assay

HEK cells are transfected with the mRNA vaccine. As a consequence, the HEK cells express the SARS-CoV-2 spike protein on their surface. After staining with both a primary and a secondary antibody, cells are analyzed by flow cytometry.

The response data (meaning the MFI) are plotted logarithmically against the respective dose of standard and sample, and sigmoidal dose-response curves are created

(using PLA 3.0). These are described mathematically with a 4-parameter fit (4-PL), and according to the parallel line method, the relative binding activities of the samples compared to the standard are calculated by the software.

The test item must be tested twice independently, resulting in two microtiter plates per sample. The combination (weighted mean) of these two test results represents the final bioactivity, and the reported value.

Schematic Overview
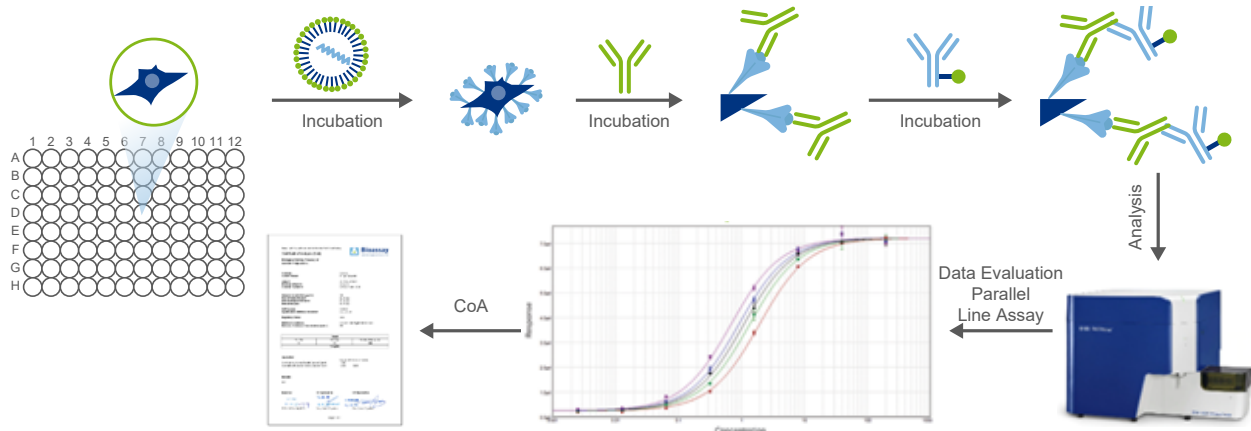
Potency Assay Example

Assay Setup and Evaluation

- Standard was used with 40% and 160% potency level as sample
- Titration was performed over 8 steps in duplicate
- Mean fluorescence intensity is shown on graph as response

- A 4-PL fit was used for potency determination
- Assay was „passed" according to Eu. Pharm. Chapter 5.3
- mRNA vaccine concentration shown on graph as relative dose (log)

Potency Estimation 40% vs. 100%

- Estimated vs Stated Potency:
  38.9% vs. 40.0%
- 95.0% Confidence Interval:
  0.371 – 0.0.408
- Relative Confidence Interval:
  95.37% – 104.86% (9.49%)
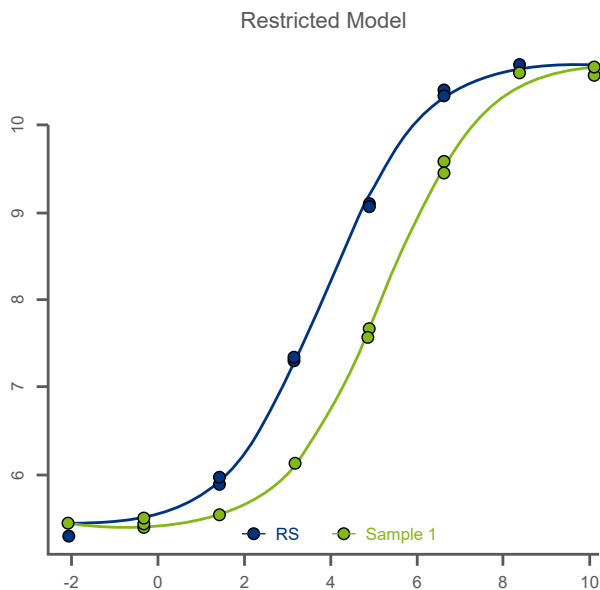
Potency Estimation 160% vs. 100%

- Estimated vs Stated Potency:
  165.1% vs. 160.0%
- 95.0% Confidence Interval:
  1.572 – 1.734
- Relative Confidence Interval:
  95.20% – 105.04% (9.84%)

Restricted Model

Restricted Model