

# Consistency with excellence: Lot-to-lot monitoring of Olink<sup>®</sup> PEA probes

## Introduction

Proximity Extension Assay (PEA) converts an antibody-based immunoassay to a robust digital DNA signal, with readout using either Next Generation Sequencing (NGS) or quantitative real-time PCR (qPCR). This results in a scalable, multiplex and highly specific method where the concentrations of thousands of protein biomarkers can be quantified simultaneously.

The PEA probes (pairs of oligonucleotide-conjugated antibodies) are critical components of the PEA kits for Olink Explore (NGS) and Target (qPCR) panels. Maintaining each assay's dynamic range and stability is of great importance.

Before release, all probes are rigorously quality controlled to ensure the quality of the conjugation, antibodies and attached oligos. The validation data is made freely available on our [website](#).

## Assessing consistency

### Calibrator curve monitoring to minimize variation over time

For Target 96 and Explore, a calibrator curve (7 points at 10-fold dilutions), a dilution series (plasma from healthy subjects) as well as reference samples (plasma from 10 patients with specific diseases) are analyzed simultaneously with each new batch and a fixed reference batch of PEA probes to address any differences

in for example dynamic range, sensitivity or detectability (Figure 1). The fixed reference batch is used to avoid drift and simplify trending of data.

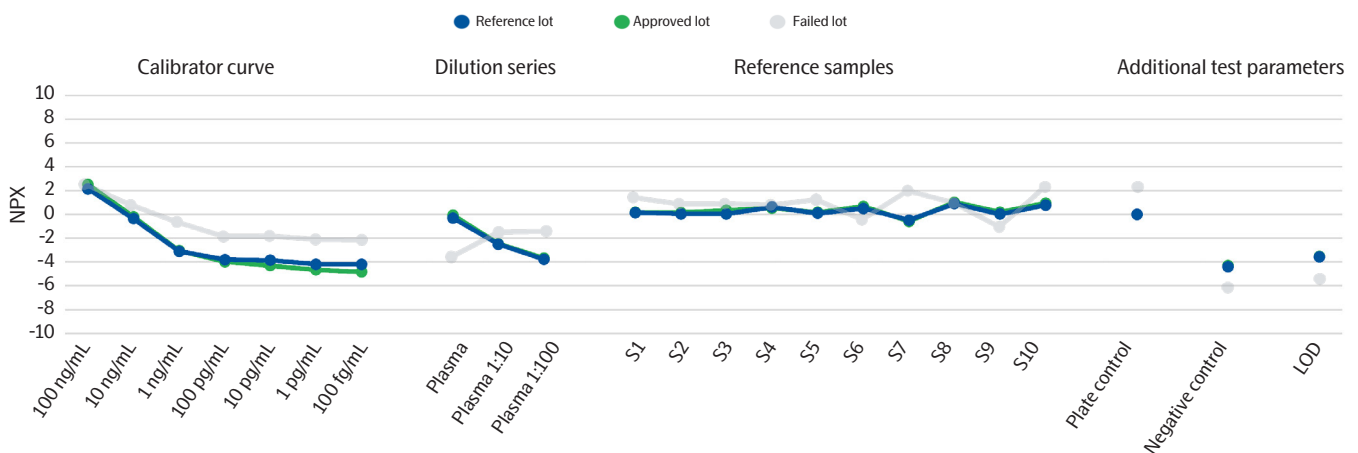
For Olink Target 48 panels with absolute quantification, the lot-to-lot variation is additionally minimized by harmonizing the output data. An 8-point calibration curve combined with a wider range of biological samples is analyzed with the two batches in multiple runs to calculate a bridging factor for each assay that will be applied in the data analysis software. This will give absolute quantification in standard concentration units (pg/mL).

If the performance of any assay is deemed affected, the assay is failed and reproduced.

### Guidelines for passing QC

The procedure for passing quality control (QC) includes the following:

- Parallel shift is evaluated and controlled. This is to check that the antigen-specific and background signals change equally, indicating there is no difference in sensitivity/detectability.
- Measuring range shift in calibrator curve is evaluated.
- Reference sample correlation is evaluated to ensure the same protein profile for each batch.
- In addition to the above, a manual inspection of each assay is performed, comparing the two batches.



**Figure 1** Illustration of the probe QC assessment. Sample and antigen response measured when comparing two probe batches in Olink Explore. They grayed out curves show a failed batch.

## Example of lot-to-lot variation assessment

Lot-to-lot variation was assessed by compiling data from four different probe batches of the Olink Target 96 Inflammation panel. In these batches, both antibody batches and conjugation rounds were varied. On average, these were the observed coefficients of variation between batches (%CV):

- Between conjugations: 17.6%
- Re-pooling of the same probe stocks: 13.1%
- New antibody batch from vendor: No significant difference compared to re-conjugation of the same batch was observed (16-19%)

## Reagent stability and shelf life

To assess reagent stability, panel and kit components are tested over time and compared to a reference. Stability data indicates consistent output for substantially longer period of time without significant change in performance. The recommended shelf life when stored under recommended conditions is listed in Table 1.

## Conclusions

Olink has implemented extensive QC procedures that allow full visibility and control over the technical performance of the assays and minimize lot-to-lot variation to help ensure the generation of reliable data that customers can trust.

Table 1. Shelf life of Olink probes and components. \*Target 48 was recently launched and a real-time stability study is ongoing to evaluate if the 18 months shelf life can be prolonged or not.

Product	Component	Shelf life (months)
Explore	Probes	30
	Generic components	30
Target 96	Probes	36
	Generic components	36
Target 48	Calibrators	18*
	Probes	18*

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