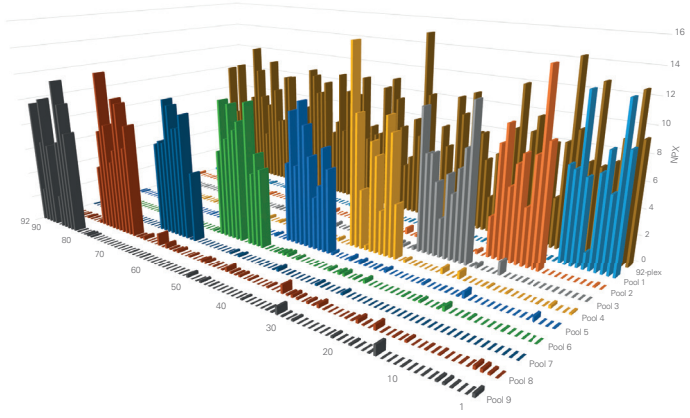
PEA: Exceptional specificity in a high multiplex format

Technology features

The dual-recognition, DNA-coupled readout provided by Olink's Proximity Extension Assay (PEA) technology provides exceptional specificity even at high multiplexing levels. For each protein target, two oligonucleotide-coupled antibodies (PEA probes) must bind in close enough proximity to enable the oligos to hybridize and form a unique DNA template for detection by qPCR. This dual antibody recognition and hi-fidelity DNA-coupled measurement mean that PEA is able to provide truly exceptional readout specificity. This overcomes the problems normally associated with multiplexed immunoassays, since any potential antibody cross-reactivity will not contribute a detection signal. This degree of specificity is a hallmark of PEA. In this document, evidence to support the PEA specificity is presented, such as Olink assay specificity, the lack of cross-reactivity, correlation with standard single-plex ELISA and orthogonal validation through genomics.

Assay specificity

In a study by Assarsson et al. *(1)*, assay readout specificity of the Olink platform was tested for one Olink panel. To ensure that the antibodies selected were specific for their desired targets, each assay response to all of the 92 proteins in the panel was measured, as well as against an additional 107 proteins (not shown). In principle, the specificity was tested by creating a test sample consisting of a pool of antigens, which was then incubated with all 92 antibody probe pairs from the panel. Only if there was a correct match would a reporter sequence be created and serve as a template for subsequent real-time qPCR. Ten sub-pools of antigen were evaluated to cover the 92 assays in the Olink panel as illustrated. None showed significant signal from the proteins tested.



Cross-reactivity

Cross-reactive events are a common problem for multiplex immunoassays, such as sandwich ELISA. The dual recognition of PEA and multiple blocking reagents included in the immunoassay step, prevent non-specific binding using Olink panels.

In one experiment, a set of highly homologous proteins were used to search for cross-reactive recognition of related proteins and to further challenge specificity *(2)*. Homologous proteins from Olink's antigen library (n ≈ 1500) were included if they had an amino acid sequence coverage ≥ 90% and/or identity ≥ 50% according to the protein database Protein BLAST. Endogenous levels were used for both specific and homologous proteins as indicated in the following table:

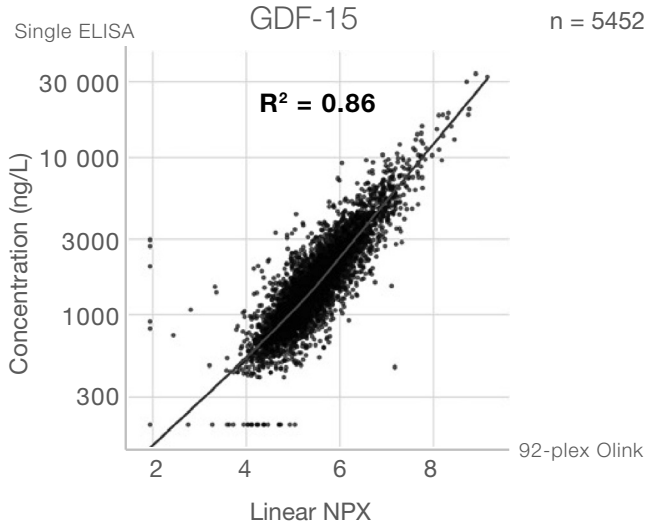
Assay	Related protein	Coverage (%)	Identity (%)	Cross-reactivity (%)
FAPB4	FABP9	99	64	0.0
FR-alpha	FR-beta	87	77	0.1
KLK11	KLK8	90	49	0.0
EN-RAGE (S100A12)	S100P	98	45	0.0
CDH3	CDH1	97	54	0.0
CDH3	CDH2	87	46	0.0
CDH3	CDH4	87	45	0.0
MK	PTN	71	51	0.0
Dkk-4	Dkk-3	90	27	0.0

Despite testing the most related proteins, cross-reactivity was not observed. FR-alpha showed some recognition of its highly homologous relative FR-beta (77% identity and 87% coverage), although at a non-significant level in plasma (0.1%). This systematic approach demonstrates that the Olink assays can distinguish between very similar human proteins, and yet again highlights the high specificity of PEA.

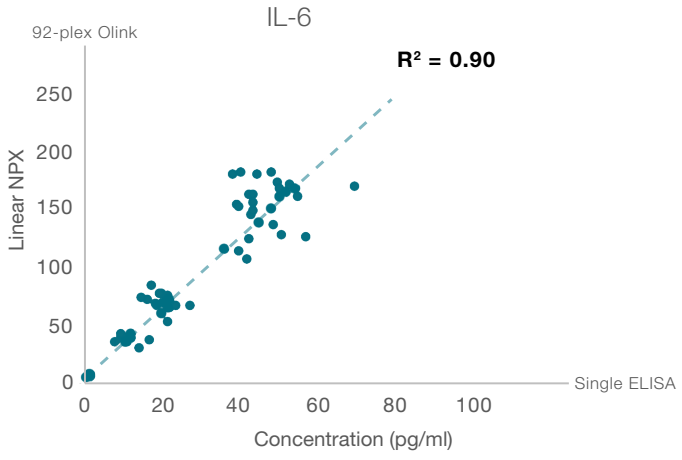
ELISA correlation

Olink's multiplex assays correlate well with standard, single ELISAs according to a study by Siegbahn et al *(3)*.

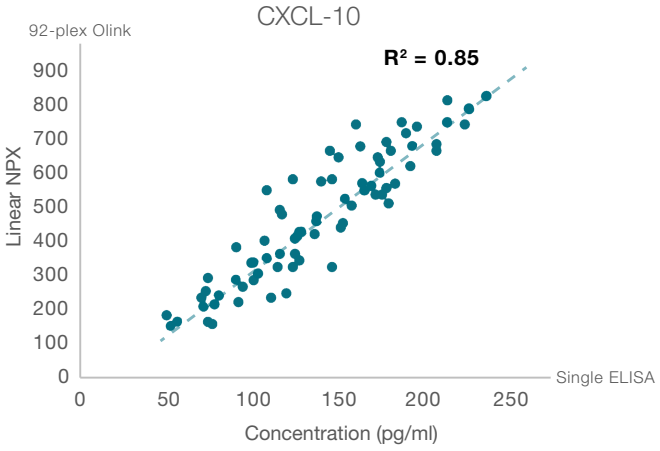
In the study, clinically approved single-plex assays were run in a total of 10 000 samples from three large cohorts. The correlation between the single-plex assays with Olink multiplex is shown below for GDF-15. Note that the Olink panel only needed 1 µl of sample.



Below are examples of direct comparisons, using the same antibodies, with commercially available ELISAs. In the first example, IL-6 was compared and a strong correlation between the Olink 92-plex PEA assay and a single-plex ELISA was seen.



One more example of similarly excellent correlation between our 92-plex results compared to single-plex ELISA is illustrated for CXCL-10.



Lack of correlation—a case study

There are occasionally cases where the data for an Olink assay does not correlate at all with a standard ELISA – so in that situation, how do we figure out which assay is correct?

Background

A problem was discovered during an Olink analysis. Aliquots of cell lysates and cell supernatants were analyzed using the Olink Inflammation panel at Olink Analysis Service and significant differences were observed between different cell groups for Leukemia inhibitory factor (LIF) and Leukemia inhibitory factor receptor (LIF-R).

When the same cell lysates and supernatants were analyzed using an ELISA for LIF and LIF-R by a customer, however, the significant results obtained by Olink could not be repeated using the ELISA.

Experiments and results

Olink carried out a series of experiments to investigate the matter further. The results from Olink's investigations indicated that the LIF-R ELISA kit the customer used, actually measures a LIF-like protein sequence and that that the Olink Inflammation panel measures LIF-R correctly. Contact support@olink.com for more information.

SCALLOP

SCALLOP (www.olink.com/scallop) is a collaborative framework for combined genomics and proteomics studies to enable discovery of protein quantitative trait loci (pQTLs) and novel biomarkers using Olink panels for the protein analysis. In one publication they reported a substantial frequency of cis-pQTLs *(4)*, which provides very strong orthogonal validation of specificity via correlation of protein levels with specific DNA changes within the genes coding for those same proteins.

References

1. Assarsson E. et al., Homogenous 96-plex PEA immunoassay exhibiting high sensitivity, specificity, and excellent scalability. PLOS ONE 9, e95192 (2014).
2. Olink White paper Development and validation of customized PEA biomarker panels with clinical utility (2017)
3. Siegbahn A. et al., A comparison of the proximity extension assay with established immunoassays, in Advancing precision medicine: Current and future proteogenomic strategies for biomarker discovery and development, p. 22-25. Science/ AAAS (2017).
4. Folkersen L. et al., Mapping of 79 loci for 83 plasma protein biomarkers in cardiovascular disease. PLOS Genetics (2017)

1113, v1.0 2019-01-30

olink PROTEOMICS

Olink Proteomics
Dag Hammarskjölds v. 52B
SE-752 37 Uppsala, Sweden
www.olink.com