Olink Explore HT Analytical Validation

Version 3.0

Mt. Sinai CIMAC

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The table below lists the nature of initial results produced (primary output), QC steps and analyses, and eventual output.

Assay type	Primary assay outputs	Pre-processing/ Normalization /QC	Initial analyses	Derived data outputs
Olink Explore HT	5,416 targeted soluble factor immunoassay	Normalized protein expression (NPX)	Multivariate Coexpression Analyses	Quantification and correlation of multiplexed cytokine levels

OLINK PROTEIN SOLUBLE ANALYTES	
(i) Olink Explore HT verification Process	Three-step validation (sensitivity, specificity, dilution linearity) process for every Olink Explore HT assay and common sample matrices (see **Figure** 2). 15,300 assays tested, 5,416 approved.
ii) technical detectability	100% of proteins detected in at least 1 sample of plasma or cell/tissue lysate origin
(ii) precision: Intra-assay and Inter-assay	Intra and inter-assay Coefficient of Variation (CV) of concordance samples for precision of the Olink Explore HT assay: 8.4% intra CV; 7.2% inter CV (**Figure** 4)
(iii) analytical sensitivity	Analytical measurement range and LOD are determined for each assay using 16-point calibration curve using 4-fold dilution (**Figure** 3)
(iv) analytical specificity	Predefined protocol with three levels of specificity testing. 99.5% of 5,416 assays exhibited no cross-reactivity
(vi) scalability	A mean correlation of >0.8 for correlation data between Olink Explore HT and Olink Target 96 (9 different panels) for over 100 study samples for overlapping analytes; Mean correlation of 0.81 and a median correlation of 0.88 between Olink Explore HT and Explore 3,072 for 2,800 overlapping analytes
(vii) standardization, harmonization, reproducibility and ruggedness	Assay standardization is established at levels of assay, operators and equipment, and Mt Sinai HIMC has been recognized as a “Certified Service Provider of Olink Analysis” by the Olink company following robust training and SOPs.
(viii) establishment of appropriate quality control and improvement procedures	HIMC uses concordance samples and in-house control sample samples (normal human plasma pooled from 15 subjects) to measure inter- and intra-assay variations. HIMC passed the concordance test between our own lab and Olink Analytical Service, and was certified by Olink since June 2017. HIMC certifies the assay providing service annually.

(ix) any other performance characteristics required for assay performance	All of the required equipment, including Formulatrix F.A.S.T., SPT Labtech DragonFly, ProFlex Thermocyclers, centrifuges, all have preventive service contracts to ensure optimal performance. All other small equipment such as mixmate, multi-channel pippetman also has biannual calibration performed by certified vendors.

1. Purpose of assay

The Olink® Explore HT platform represents a cutting-edge advancement in high-throughput protein biomarker discovery. It stands out due to its ability to analyze approximately 5,400 proteins from just 2 µL of sample, making it both efficient and scalable. Olink Explore HT library expansion has significantly broadened its scope to cover a diverse range of proteins involved in various biological processes. By including more inflammatory, immune response-related, secreted, drug target, disease-associated, and canonical plasma proteins, this expansion enhances the library's ability to provide comprehensive insights into the human proteome.

2. Technology, assays performance characteristics and Olink's internal validation

Olink's innovative Proximity Extension Assay (PEA) Technology uses multiplexed oligonucleotide-labeled antibody probes. Each probe pair is designed to bind specifically to its target protein in the sample. When these antibody pairs bind closely together at their target protein, the oligonucleotide sequences on the antibodies come into close proximity, which allows them to hybridize and form a PCR reporter sequence through a DNA polymerization event. The PCR reporter sequence is then amplified through PCR. This amplified DNA barcode is detected and quantified using Next Generation Sequencing (NGS), a highly sensitive method for reading and quantifying DNA sequences. The protein concentrations are calculated using specialized processing software. Results are provided in NPX (Normalized Protein eXpression), a relative concentration unit that facilitates the comparison of protein levels across samples and experiments. The Olink assay is compatible with various specimens, such as serum, plasma, CSF, cell/tissue lysate, DBS (Dried blood spot), EV (extracellular Vesicles), Lavage supernatant.

2.1 Quality Controls

Internal and external controls have been developed by Olink for data normalization and quality control. These have been designed to enable monitoring of the technical performance, as well as the data quality of individual samples, providing information at each step of the Olink protocol (**Figure 1**).

Three internal controls are added to each sample: Immuno Control, Extension Control and Amplification Control. The Immuno Control (an assay targeting a non-human antigen) monitors all three steps starting with the immuno reaction. The Extension Control is an antibody linked to two DNA tags in close proximity that provides a constant signal independent of the immuno reaction. This monitors the extension and readout steps and is used for data normalization across samples. Finally, the Amplification Control (a synthetic double-stranded DNA template) monitors the amplification and readout steps. Internal controls are mainly used to monitor the run quality at sample level, including both customer samples and external control samples.

Three different types of external controls are included in the protocol. Negative Control is included in duplicate on each plate and consists of buffer run as normal sample to assess potential contamination of assays. Plate Control is included in 5 replicates on each plate. The median of the Plate Control replicates is used to normalize each assay and compensate for potential variation between runs and plates. Sample Control is included in triplicate on each plate. These samples are used to assess potential variation between runs and plates, for example to calculate inter-assay and intra-assay CV, as well as for troubleshooting.

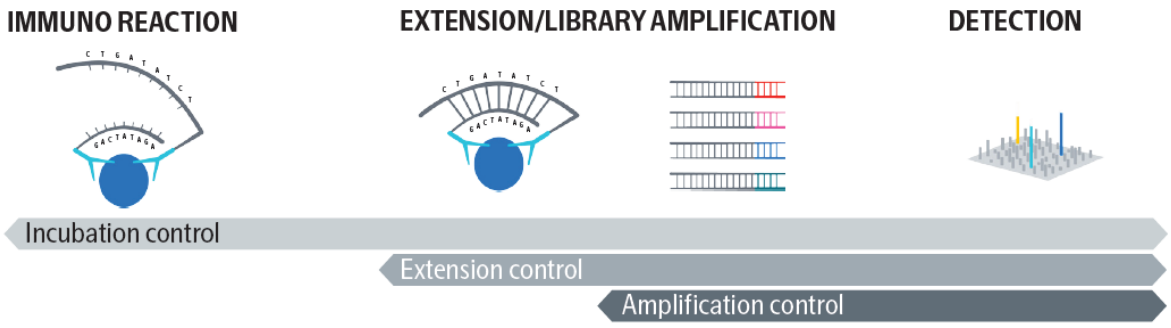

Figure 1. Olink assay procedure. The inter controls enable monitoring of the three core steps of the method and are used for quality control

2.2 Data pre-processing and normalization

Preprocessing is a necessary step in all NPX Explore analyses. During preprocessing, the Next Generation Sequencing (NGS) output from the sequencing instrument is converted to counts files containing the number of reads for each Olink sequence and a run metadata file containing additional information which is necessary for importing the counts files into the analysis software. NPX™ Explore HT & 3072 is designed for Olink® Explore platforms. It allows for importing data, validating data quality, and normalizing Olink data for subsequent statistical analysis. Quality control is performed by trained users who determine whether data from a run can be approved for further analysis. Data that have passed QC undergo normalization and NPX values are generated. The NPX values are calculated in two main steps. The ~5,400 biomarkers in the library are arranged into 8 blocks depending on protein abundance and sample dilution factor as described in the Olink Explore HT User Manual. First, the counts of each biomarker from a sample are divided by the Extension Control count for that sample and block. A log2 transformation is then applied. In the last step the median of the Plate Controls is subtracted for each biomarker.

There are two between-plate-normalization methods: Plate Control (PC) normalization and intensity normalization. They both adjust each assay per plate to a median, but differ in how these medians are calculated. Plate Control normalization should be selected for all Explore HT project (projects with single or multiple plates), when complete randomization of samples cannot be guaranteed. Plate Control normalized NPX will by default be reported in the output NPX file. For projects with samples well-randomized between plates, intensity normalization should be used, which sets the median level of all assays to the same value for all plates.

2.3 Olink Explore HT verification process

Every assay included in Olink Explore HT undergoes a stringent 3-step analytical verification process. In the first step, the protein biomarkers are tested using a 96-plex format with qPCR readout. If the biomarker passes all test criteria in step one, it is allowed to move on to the second step where a 192-plex format with NGS readout is used. The biomarker assays passing all test criteria in the first two steps, are incorporated into the Olink Explore HT panel. In the final step, each protein biomarker is thoroughly tested together using the final panel design.

To determine if a biomarker passes the test, sensitivity, specificity, sample distribution for commonly used sample matrices (plasma, serum, CSF and tissue and cell lysate samples) as well as sample dilution linearity are tested with defined acceptance criteria. The tests and acceptance criteria for steps 1 and 2 are very similar. The main differences are the plex grade and readout method, as described above. An examples of verification testing is shown in **Figure** 2. The sensitivity test used a calibrator curve. A calibrator curve is defined using recombinant antigens (from various sources including E. coli and mammalian), to evaluate the sensitivity and measurement range of the assay. Biomarker B passed with wider detection range (with detection above 1:100,000 dilution) based on known concentrations of the recombinant antigen.

Specificity test pools are used to identify and exclude biomarkers with non-specific binding. Here the goal is to detect the intended antigen only (all other sub pools should have NPX levels below LOD). The test is repeated, and similarly only one sub-pools should be identified. If several peaks are identified, the assay may be cross-reacting, indicating it detects signal contributions from more than one antigen. Biomarker A has noisy basal level and detected antigen in the wrong peak. In contrast, Biomarker B detects intended antigen of different sub-pools with measurement at the same concentration, with all other sub-pools below LOD.

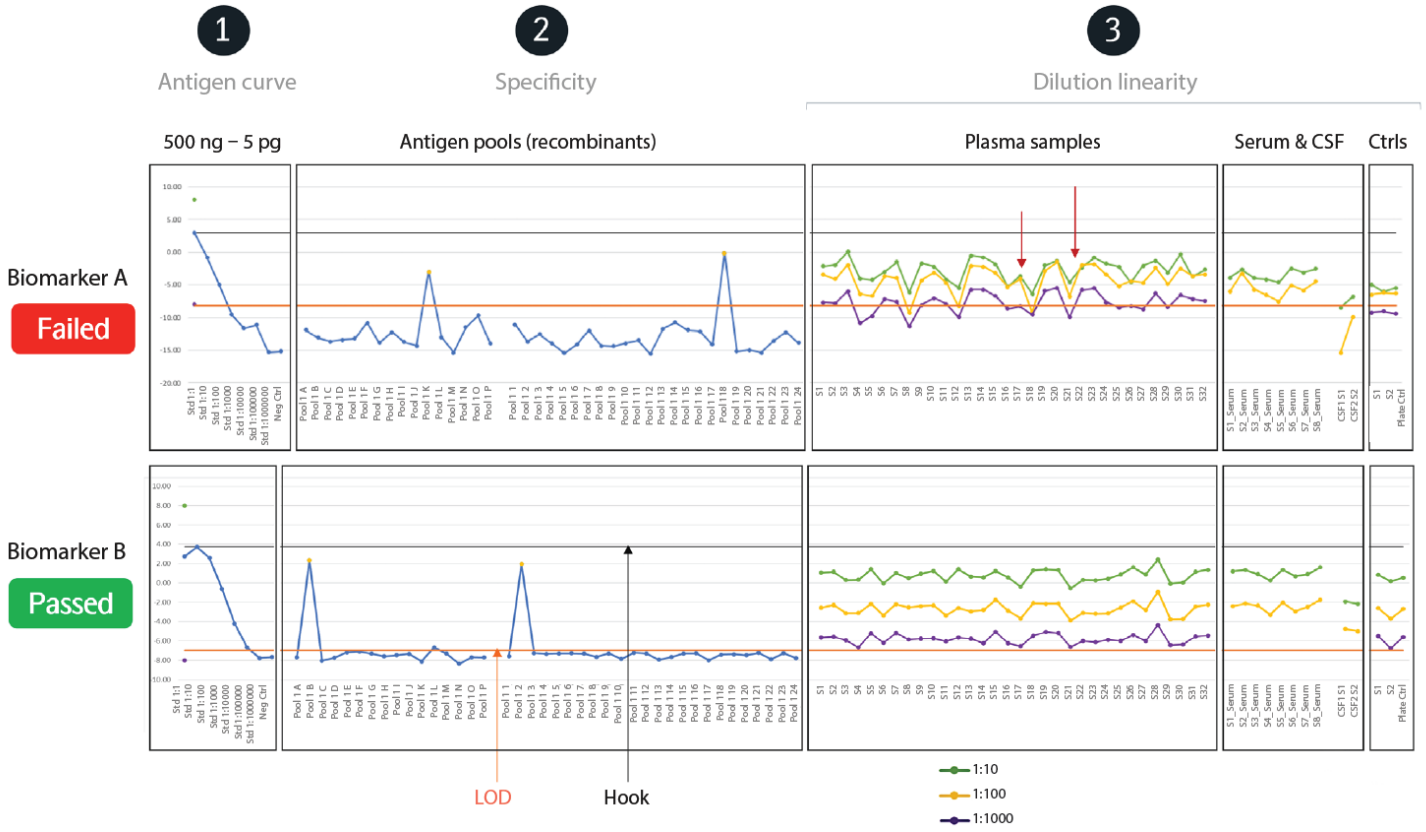

Figure 2. Example of NGS screening results. 1) Calibration curve. 2) specificity test. 3) Dilution linearity

For dilution linearity, the assay performance and expected NPX levels in real samples, a range of plasma, serum and CSF (cerebral spinal fluid) samples from diseased and healthy individuals, as well as Explore HT Control Samples are tested. These samples are assayed in 3 different dilutions. Here the goal is to verify that all samples dilute well and that the NPX level pattern is reproducible across dilutions. As in Figure 2, Biomarker A did not demonstrate dilution linearity for plasma, serum, CSF and Olink control samples.

As mentioned above, the biomarkers that passed the test in step 1 and 2 were then thoroughly tested together in the final Olink Explore HT panel design. The results and methods for step 3 are described in the following sections of Olink internal validation.

2.4 Sample Information

Both recombinant antigens and commercially available EDTA plasma samples were used in Explore HT validation to evaluate the sensitivity, precision, specificity and scalability. The plasma sample included healthy subjects (n=21) and patients (n=80) with a range of diseases, including cardiovascular (n=20), autoimmune/inflammatory (n=20), neurological (n=20) and cancer (n=20). Most of the disease samples had multiple diagnosed conditions. External control samples were placed on each plate in all tests.

2.5 Sensitivity

To determine the analytical measurement range for each assay, 16-point calibration curves were created using 4-fold dilutions of the antigens, starting with an initial antigen concentration of 500 mg/mL. The uppermost point of the calibration curve is referred to as Hook. The measurement range for each protein is defined as from Limit Of Detection (LOD) to Hook-1NPX, or if Hook is not reached, as LOD to the uppermost point in the calibration curve. The LOD is calculated based on the Negative Controls (NC) in the validation runs. LOD is defined as 3 standard deviations (SD) above background and reported in NPX. LOD can be calculated in two ways, and whichever value is higher is used.

LOD = Median (NCs) + 3SD (NCs) or Median (NCs) + 0.2

If a low sequencing signal is generated, i.e., the maximum number of counts in NCs ≤ 150 counts, the LOD is set to a counts level using a read count threshold. The read count threshold is equal to 2 times the maximum counts in NCs (or 150, whichever is highest).

Olink Explore HT offers a broad dynamic range spanning over 10 - 12 orders of magnitude, allowing the detection of high to very low abundance proteins (mg/mL to fg/mL). The high dose Hook effect is seen when there is an antigen excess relative to the reagent antibodies, resulting in incorrectly underestimated results. In such cases, a significantly lower value may lead to erroneous interpretation of results. Additional information is reported in the Olink Explore HT data sheet, indicating whether the hook is reached. Two examples of calibrator curves with their corresponding measuring ranges are shown in Figure 3.

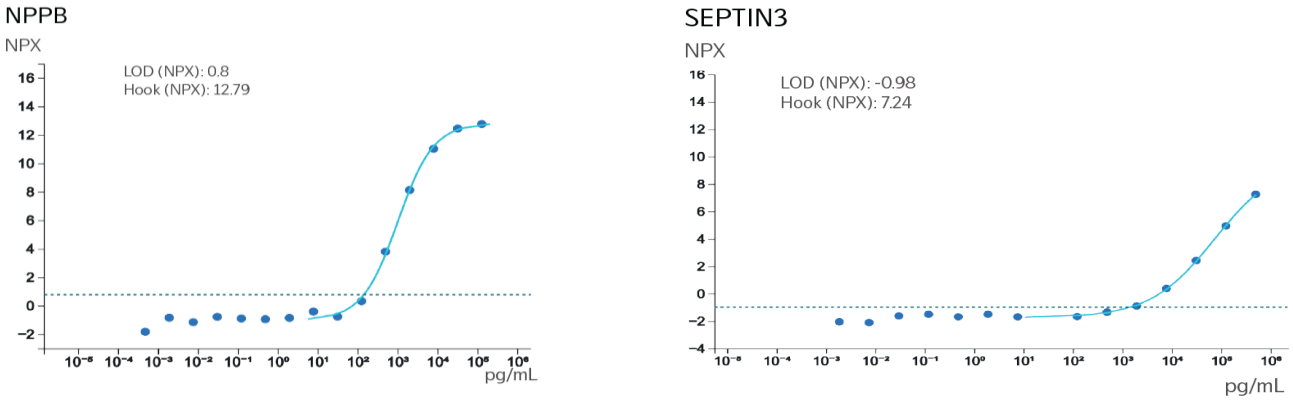

Figure 3. Calibration curve for assay NPPB (UniProt: P16860) has reached hook. Calibration curve for assay and SEPTIN3 (UniProt: Q9UH03) has not reached hook.

2.6 Precision

Intra (within-run) variation was calculated as the mean coefficient of variance (CV) for 6 individual Sample Controls, within each of 3 separate runs. Inter variation (between-runs) was calculated as the mean CV, for the same 6 Sample Controls, among 3 separate runs. The distribution of both intra- and inter- CVs are shown in **Figure** 4a and 4b.

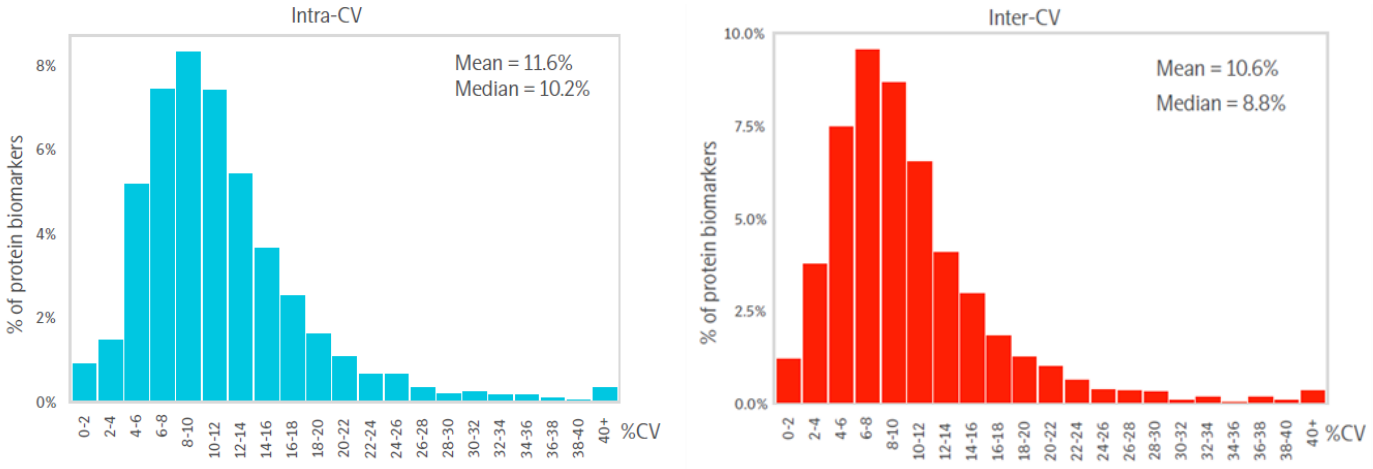

Figure 4a. Distribution of protein markers by intra- and inter- CVs

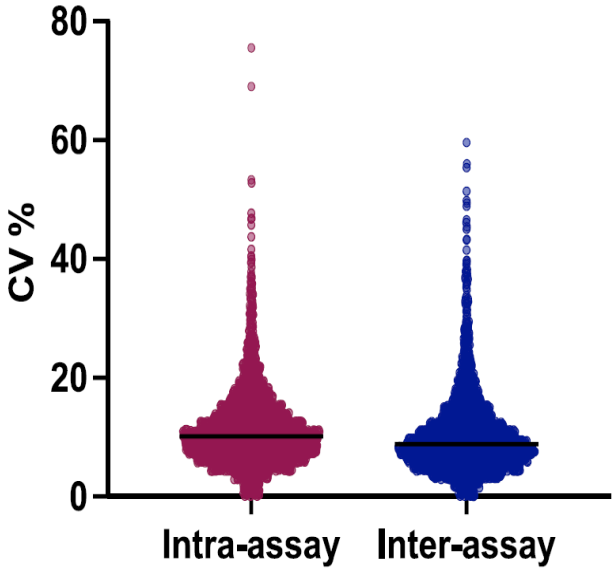

Figure 4b. Mean intra- and inter-assay CV (%)

The median intra-CVs and inter-CVs observed are 10.2% and 8.8%, respectively (**Figure** 4b).

2.7 Specificity

All Explore HT assays have gone through a predefined protocol with three levels of specificity testing. The third specificity test on the Explore HT product is described below.

In practice, it is not possible to test all Explore HT protein assays against each other to evaluate potential cross-reactivity. Here an alternative approach was used. First, all protein families in Explore HT were evaluated with regards to their amino acid homology with other proteins of the same protein family. The UniProt database was used to define the family of each protein, and the homology (% sequence identity) was determined with Python (Pairwise2) using full length protein sequences. Then, 179 antigens with high homology (over 80%) were selected to create the specificity test pool in order to investigate potential signal contribution from closely related proteins. The specificity test pool was divided into 64 sub-pools, each antigen was present in two different pools. All pools were diluted to 50 and 5 ng/mL and tested with Explore HT twice. If 5 ng/mL of antigen was detected in both pools and antigen with 50 ng/mL gains higher signal than 5 ng/mL, the detection was considered to be true and specific.

In total, 99.5% of analytes (5389/5416) in Explore HT exhibited no cross-reactivity according to the tests described above. A total of 27 analytes revealed a cross-reactive signal, meaning a signal contribution from a different protein. In all cases, this was to a closely related member within its protein family. The results for the 27 analytes where cross-reactivity was observed are presented in the Table 1. Note that the in vivo concentrations of the protein biomarkers may be much higher or lower than the concentration tested (5 ng/mL). Therefore, the signal contribution at endogenous level may differ between proteins and individuals.

Protein X		Protein Y		Sequence homology	Estimated signal contribution
Uniprot	Gene name	Uniprot	Gene name		
P05187	ALPP	P10696	ALPG	96.4%	~25%
P10696	ALPG	P05187	ALPP	96.4%	~50%
Q08708	CD300C	Q9UGN4	CD300A	80.4%	~12%
Q9UGN4	CD300A	Q08708	CD300C	80.4%	~25%
P41208	CETN2	Q12798	CETN1	83.7%	50-70%
P0DN86	CGB3_CGB5_CGB8	P01229	LHB	85.2%	25-50%
P01241	GH1	P01242	GH2	93.1%	~12%

Q86SG3	DAZ4	Q9NQZ3	DAZ1	100.0%	~12%
Q9NQZ3	DAZ1	Q86SG3	DAZ4	100.0%	~100%
Q6ZTU2	EP400P1	Q96L91	EP400	84.2%	~12%
P31994	FCGR2B	P12318	FCGR2A	92.0%	~25%
P12318	FCGR2A	P31994	FCGR2B	92.0%	~50%
Q6NT46	GAGE2A	O76087	GAGE7	96.6%	~17%
P62760	VSNL1	Q9UM19	HPCAL4	89.5%	~25%
O95239	KIF4A	Q2VIQ3	KIF4B	93.8%	~12%
Q2VIQ3	KIF4B	O95239	KIF4A	93.8%	~35%
P02533	KRT14	Q04695	KRT17	82.6%	~25%
Q8NFP7	NUDT10	Q9NZJ9	NUDT4	90.2%	<25%
Q6P474	PDXDC2P	Q6P996	PDXDC1	97.0%	~12%
A6NI47	POTEM	B2RU33	POTEC	86.6%	~18%
B2RU33	POTEC	H3BUK9	POTEB2	90.3%	~25%
Q96PQ5	PPP1R2P1	Q6NXS1	PPP1R2B	87.8%	~100%
P63098	PPP3R1	Q96LZ3	PPP3R2	84.7%	~100%
P05451	REG1A	P48304	REG1B	86.7%	~25%
Q96AT9	RPE	Q2QD12	RPEL1	96.1%	~100%
Q9NY46	SCN3A	Q99250	SCN2A	87.0%	~25%
P12829	MYL4	P08590	MYL3	82.4%	~12%

Table 1. All biomarkers with observed cross-reactivity where the antibody probe for Protein X recognizes Protein Y, and where Protein Y gives a signal contribution of at least 10%

2.8 Endogenous interference

Endogenous interference from heterophilic antibodies, e.g. human anti-mouse antibody (HAMA), and rheumatoid factor are known to cause problems in some immunoassays. Evaluation of the potential impact of this specific interference was investigated during the validation of previous panels. No interference due to HAMA or RF could be detected for any of the samples in previously tested panels, indicating sufficient blocking of these agents (data not shown). The potential impact of bilirubin, lipids and hemolysate, known interfering plasma and serum components, were evaluated at different added concentrations. An example of hemolysate levels tested is shown in Figure 5. These additions represent different patient health conditions and/or sample collection irregularities. Interference by bilirubin and lipids has previously been evaluated, and disturbance has only been observed at extreme levels corresponding to 8 or 10 times normal values, and therefore not performed for Olink Immuno-Onc I. In 14 out of 92 analytes, altered signal was observed by the addition of hemolysate. The reason is most likely due to actual analyte leaking out of the disrupted blood cells.

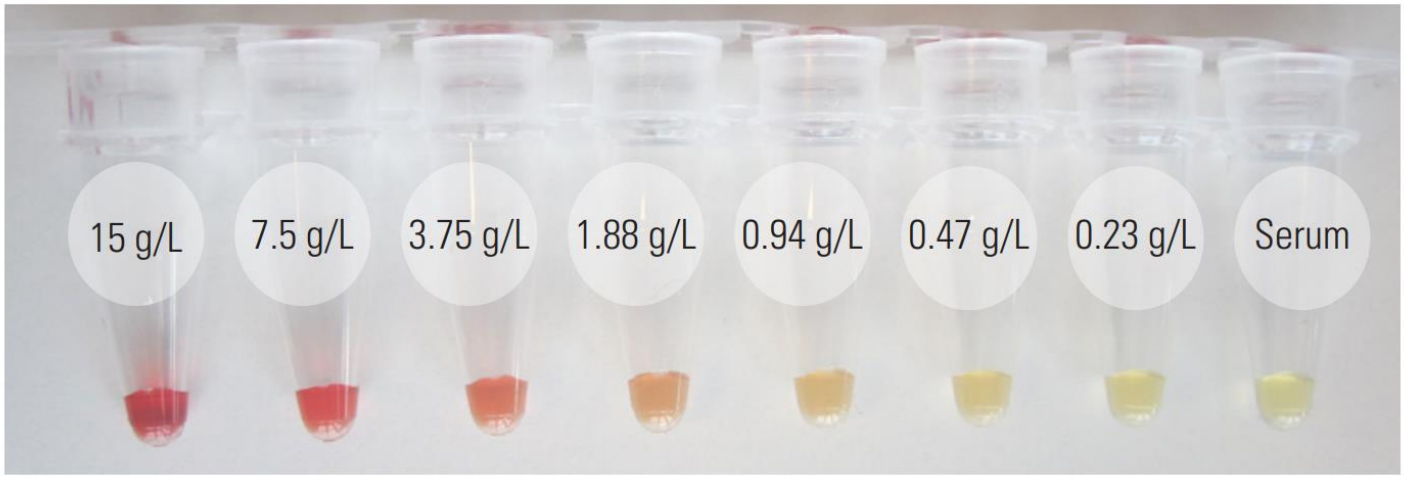

Figure 5. Endogenous interference. Levels tested for hemoglobin are 0.23 - 15 g/L. The highest concentration translates to about 10% hemolysis.

For actual proteomic assays that HIMC perform, there is stringent sample QC process. The technical specialist and operator will note any specific samples that has hemolysis, turbidity, or other visible aberration, and incorporate the information in the data report, so the study group will treat the relevant data points with caution. If the sample manifest contains any lab test result such as elevated bilirubin level, it will be noted as well.

2.9 Scalability

To compare the performance of Explore HT with Explore 3072 panels, 116 samples were run on both products in 3 repeated runs. Pearson correlation coefficient was calculated for each of the 2839 overlapping protein biomarkers using mean value of 3 replicates.

The correlation of assays for one protein (IL-6), as well as the distribution of correlation coefficients for all overlapping assays are shown in Figure 6. The median correlation coefficient (R) is 0.88. Proteins with lower correlations tended to have narrow dynamic spread among the samples tested and/or were close to the limit of detection.

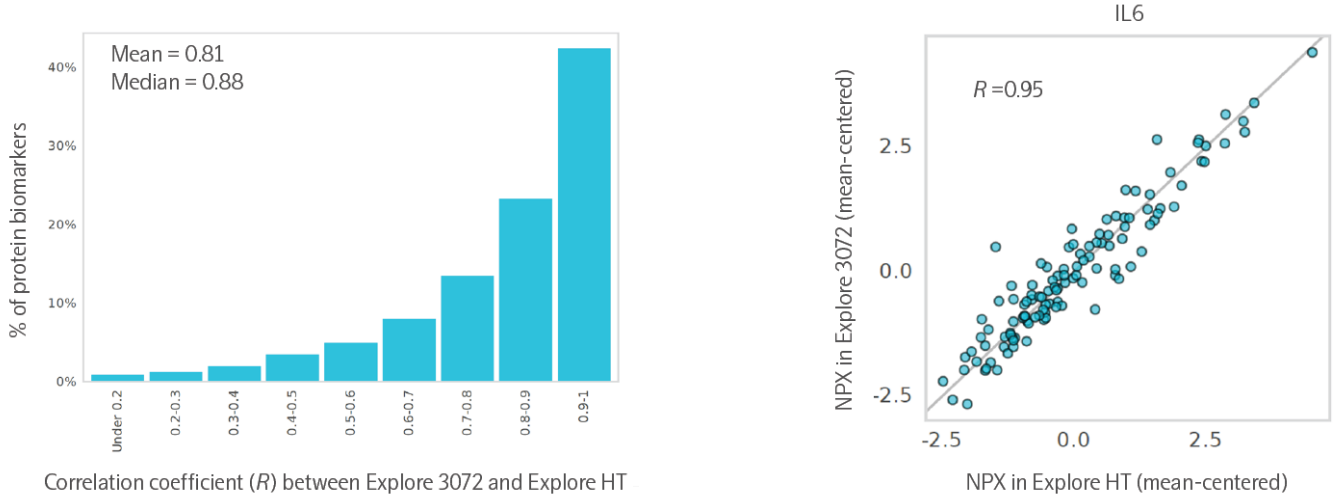

Figure 6. Distribution of correlation coefficient (left). NPX correlation plot showing the results for IL6 on Explore 3072 versus Explore HT (right).

The concordance has also been studied on real patient samples in an ovarian cancer study. As seen in Figure 7, the correlation is around 0.9 between Explore 3072 and Explore HT. This data correlation further supports the reliability of Explore HT assays in practical applications.

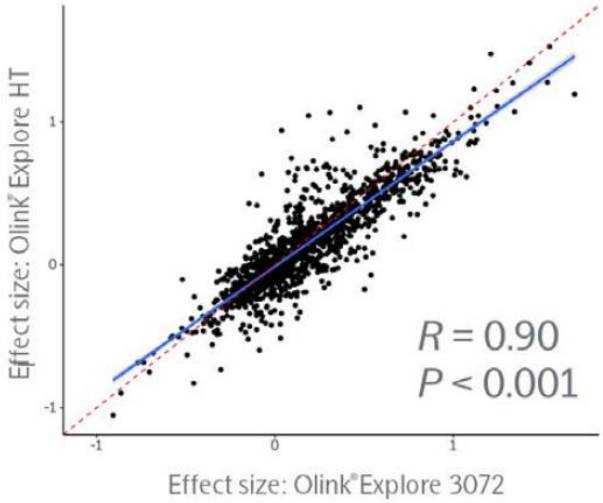

Figure7. Correlation of ΔNPX (effect size) between benign and ovarian cancer groups

Additional data proving Olink robustness and scalability came from the same concordance study, which also included two Olink® Target products. The study spans the Olink portfolio, from Explore HT down to absolute quantification with Olink® Target 48. Correlation example is shown in **Figure 8 for CXCL8**.

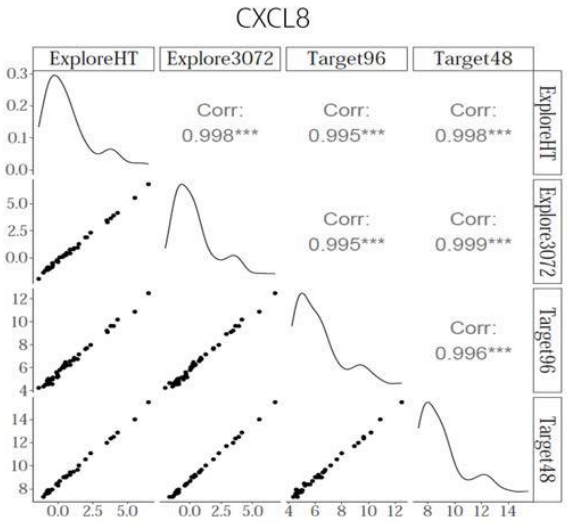

Figure 8. Representative concordance test run across Olink's portfolio showing strong concordance

Note:

1. *NPX cannot be transformed to pg/ml using the calibrator curves form the Olink in house validation curves.*
2. *The sensitivity of each protein/analyte is approximately estimated from the antigen-vendor's specification. This is not an exact concentration and needs to be treated with caution.*
3. *Recombinant antigens might not always reflect the native confirmation of proteins in plasma, which can further add to discrepancies between values obtained with antigen and native samples*
4. *A small parallel shift in NPX between batches, instruments can give rise to large differences in absolute concentration due to the sigmoidal shape of the calibrator curves*

The scalability of the assay is further addressed by including Olink panels ranging from the smallest 48-plex and 96-plex to the largest Explore HT panel with overlapping assays. A new table (Table 2) has been added, showing 92 common proteins between the Explore HT and 96-plex panels and 45 common proteins between the Explore HT and 48-plex panels, with correlation coefficients (R) exceeding 0.8. A new figure (Figure9) illustrates correlation plots comparing assays between Explore HT and the 96-plex panel.

Products	**No. of Overlapping Proteins**	**No. of Proteins with R>0.8**
Target 96 vs Explore HT	**92**	**73 (81%)**

Target 48 vs Explore HT	**45**	**37 (88%)**

Table 2. Summary of Overlapping Assays for Explore HT and Target 96 vs Target 48

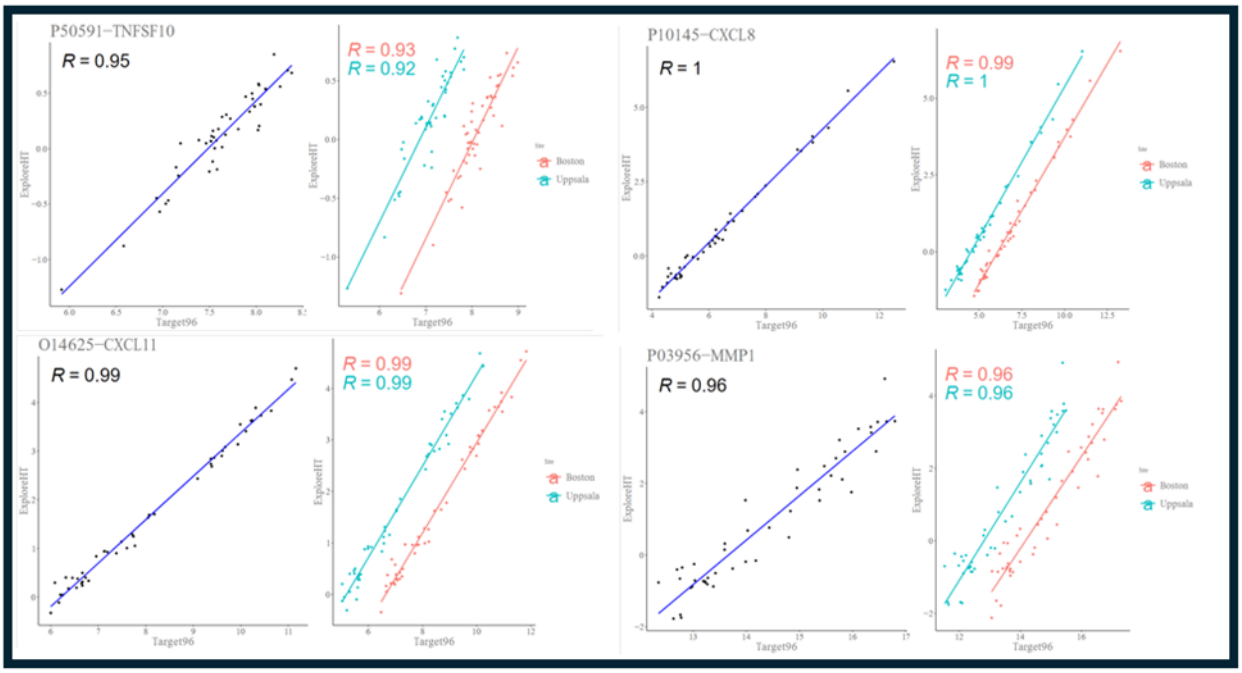

Figure 9a. Correlations Plots of Assays Comparing Explore HT and Target 96

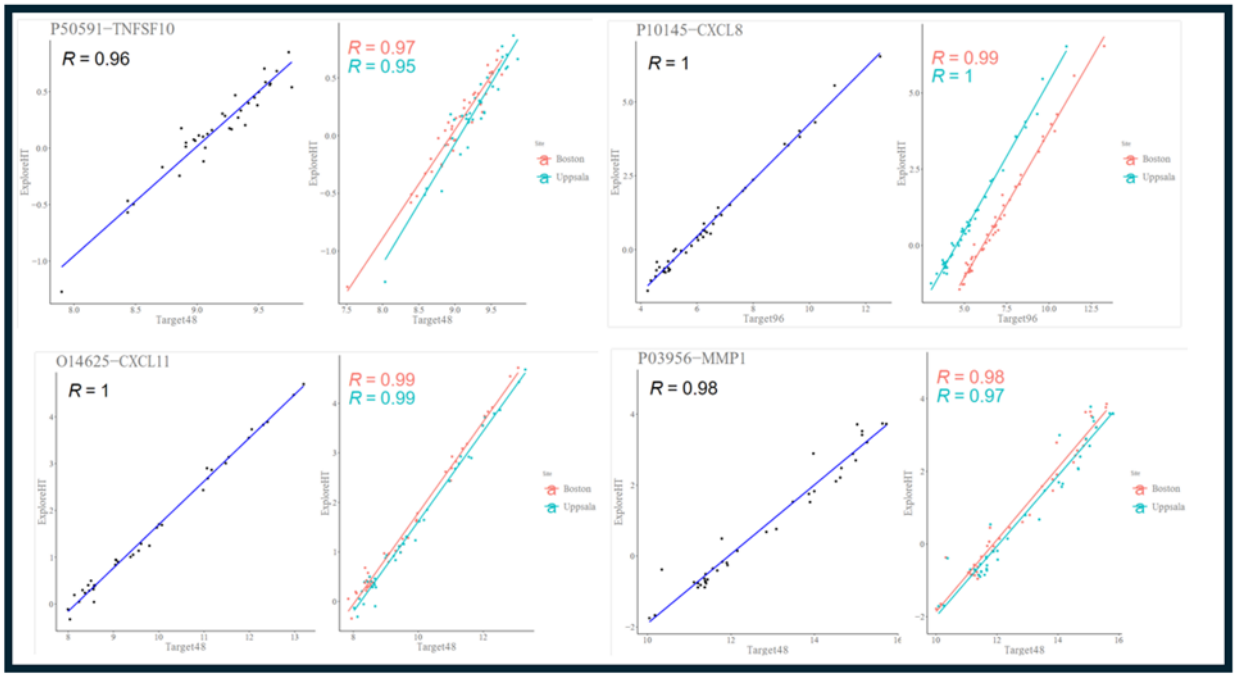

Figure 9b. Correlations Plots of Assays Comparing Explore HT and Target 48

3. Assays performance characteristics based on Mt Sinai HIMC's own internal validation

In addition to characteristics provided by manufacturer above, Mt Sinai HIMC has independently performed a series of analytical validations, as required for proficiency testing to be considered a certified user with Olink, as well as for internal documentation and correlation analysis between Olink Explore HT and various Olink Target 96 panels.

3.1 Analytes

The list of 5416 analytes in Olink Explore HT are presented in Explore HT validation data file. The lists of analytes in 9 different Olink Target 96 panels (92 proteins per panel) are shown in Table 3.

Panel Name	Analytes
Olink Target 96, Inflammation	https://olink.com/products/olink-target-96; Inflammation tab
Olink Target 96, Immuno-Oncology	https://olink.com/products/olink-target-96; Immuno-Oncology tab
Olink Target 96, Immune Response	https://olink.com/products/olink-target-96; Immune Response tab under Immunno-Oncology
Olink Target 96, Organ Damage	https://olink.com/products/olink-target-96; Organ Damage tab under Biological Process
Olink Target 96, Metabolism	https://olink.com/products/olink-target-96; Metabolism tab under Biological Process
Olink Target 96, Neuro Exploratory	https://olink.com/products/olink-target-96; Neuro Exploratory tab under Neurology
Olink Target 96, Development	https://olink.com/products/olink-target-96; Development tab under Biological Process
Olink Target 96, CVD II	https://olink.com/products/olink-target-96; CVDII tab under Cardiovascular
Olink Target 96, CVD III	https://olink.com/products/olink-target-96; CVDIII tab under Cardiovascular

Table 3. List of Olink Target 96 panels and proteins

3.2 Technical platform(s)

The Olink Explore HT runs on Olink Explore HT protocols on Formulatrix F.A.S.T., SPT Labtech Dragonfly Discovery, and ProFlex 384-well theromocyclers. The libraries are sequenced using NovaSeq X (Illumina), 10B flow cell. Olink Target 96 platform runs on Olink Signature Q100, and PCR thermalcyclers. Instruments are calibrated and serviced at least once a year. Other small instruments such as automated pipetters are also serviced regularly.

3.3 Specimens

48 concordance samples (blinded; provided by Olink) were provided by Olink and used to evaluate the HIMC operator performance and inter-laboratory variability. 88 to 115 plasma samples from cancer patients, 5 individual health donor plasma samples, 1 pooled plasma from healthy donors have been assayed across Olink Explore HT and Olink Target 96 panels to evaluate the concordance between Olink Explore HT and previously validated Olink Target 96 platform.

3.4 Assay validation data

3.4.1 Inter-laboratory variability

Inter-laboratory variability was handled by concordance samples shared between Olink and Mt Sinai HIMC as part of the certification. HIMC performed Olink Explore HT assay on concordance samples, and the concordance test result is independently evaluated by Olink Explore HT specialists and statistician to ensure HIMC operator's accuracy and proficiency in the assay. To assess the performance between the sites, Olink Concordance Test compared CV, correlation and regression between HIMC in New York (NY) and Olink Analytical Service in Boston. Because Olink Analytical Service (AS) is the industry standard among all sites for Olink assays, the comparison between HIMC and Olink AS not only assesses inter-site variability but also demonstrates the assay quality of HIMC Olink Explore HT platform. After evaluation of the results, HIMC NY passed the Concordance Test.

Scatter plots along the first two principal components were generated to compare global data performance (see Figure 10). Figure 10 displays PCA plots of AS Boston and HIMC NY data separately.

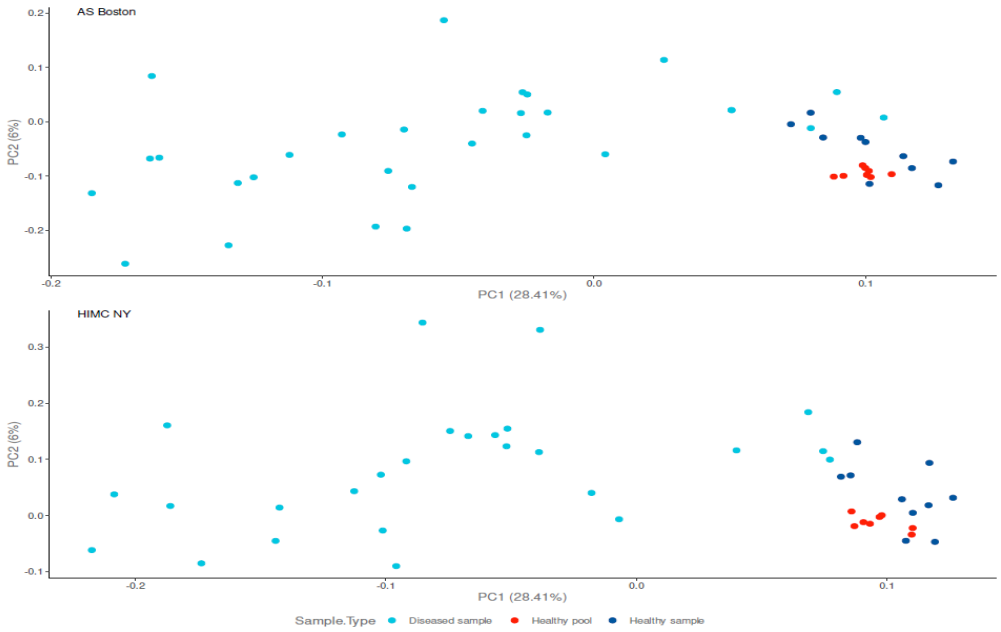

Figure 10. Separated PCA plots of data from AS Boston and HIMC NY, colored by sample type. Each point represents one sample. The position of the point is based on all measured protein values. The percentages displayed show the percentage of explained variance per principal component.

The coefficient of variance (CV) was calculated on a per assay basis, using the control samples that were included in each run (Table 4). Intra CV less than 15.0% is preferred. Correlation of NPX values between AS Boston and HIMC NY were calculated on a per assay basis (Figure 11 and Figure 12).

Olink Analytical Services at Boston	8.2
HIMC	9

Table 4. Average intra CV by site (%)

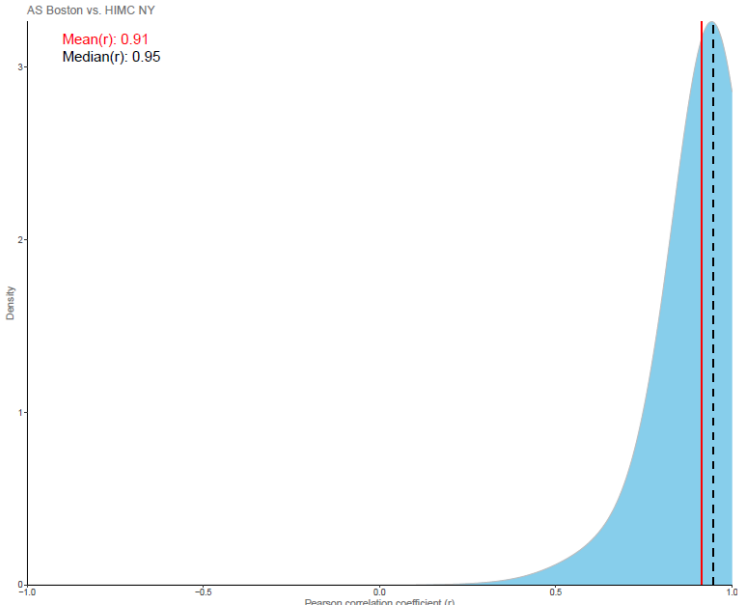

Figure 11. Density plot of Pearson correlation coefficients between two sites. The solid red line represents the mean of the correlation coefficients, serving as a central reference point, while the dashed black line indicates the median, providing insight into the distribution's central position. This **Figure**provides a powerful tool for understanding complex interdependencies within the dataset, facilitating the identification of underlying patterns and trends in variable relationships.

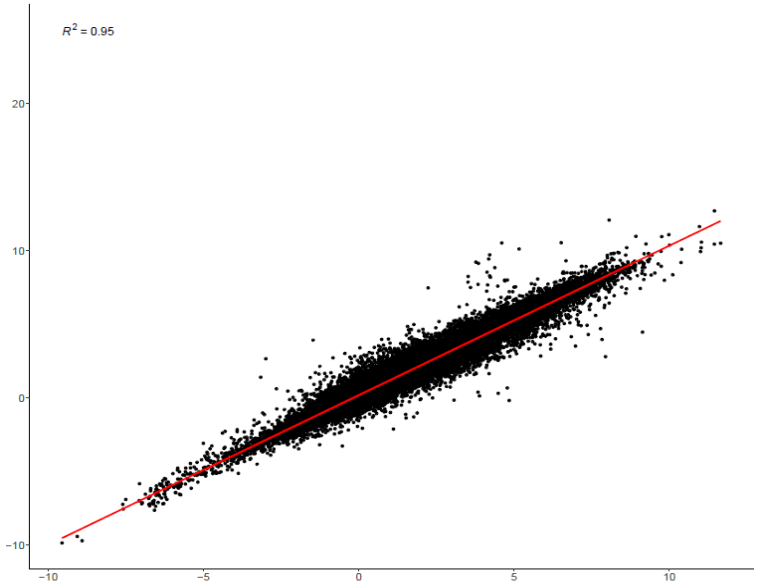

Figure 12. Scatter plot of data from both sites with a regression line fitted to present the linear relationship between two sites. In addition to the best-fit line, the **Figure**includes the R-squared (*R*2) value, which quantifies the goodness of fit of the regression model.

3.4.2 HIMC inter-assay variability

HIMC has tested 6 in-house controls that expected to have dynamic expressions in 5 different Olink HT runs. The assays were carried out by different operators. The median of protein expression of all analytes for each of the 6 samples was evaluated and is consistent between the 5 assays (as in Table 5), with highest CV% less than 15%, and average at 8.83%.

HIMC in-house control	CV%, Median of all analytes between 5 runs
Stim_Pool	5.82
Stim_HD_Pool_H	6.06
Stim_HD_Pool_M	8.38
HD_Pool_0824	14.83
HD_Pool_0824_H-Spiked	7.44
HD_Pool_0824_M-Spiked	10.5

Table 5. Average inter CV (%) for 5 runs

3.4.3 HIMC intra-assay variability and precision

124 plasma samples (from 115 cancer patients, 5 individual health donor, 1 pooled plasma from healthy donors) were assayed using Olink Explore HT. The CV for each block was assessed by NPX values of selected (5%) of the analytes among replicates of sample controls, per Olink protocol. The CV% below 25% is considered acceptable. The average of intra-assay and inter-assay CV is at 10.7% and 8.9%, respectively. See Table 6.

Block	# of assays	Dilution factor	Intra-assay %CV mean	Inter-assay %CV mean
1	742	1:1	23.3	20.7
2	1314	1:1	13.3	11.8
3	1204	1:1	9.8	7.1
4	1106	1:1	7.2	3.5
5	582	1:10	6.6	3.8
6	270	1:100	5.6	5.3
7	134	1:1000	11	6.2
8	68	1:100,000	8.6	12.4

Table 6. CV (%) of sample controls

3.4.4 Scalability

88 plasma samples from individual cancer patients were assayed in both Olink Explore HT and Olink Target 96 platforms (9 different panels as in Table 3). The concordance for common samples and overlapping analytes between two platforms were evaluated. The average correlation for z-scored NPX is over 0.8 (Figure 13). Figure13 are representative graphs. Correlations between Olink Explore HT and 7 other Target 96 panels are comparable.

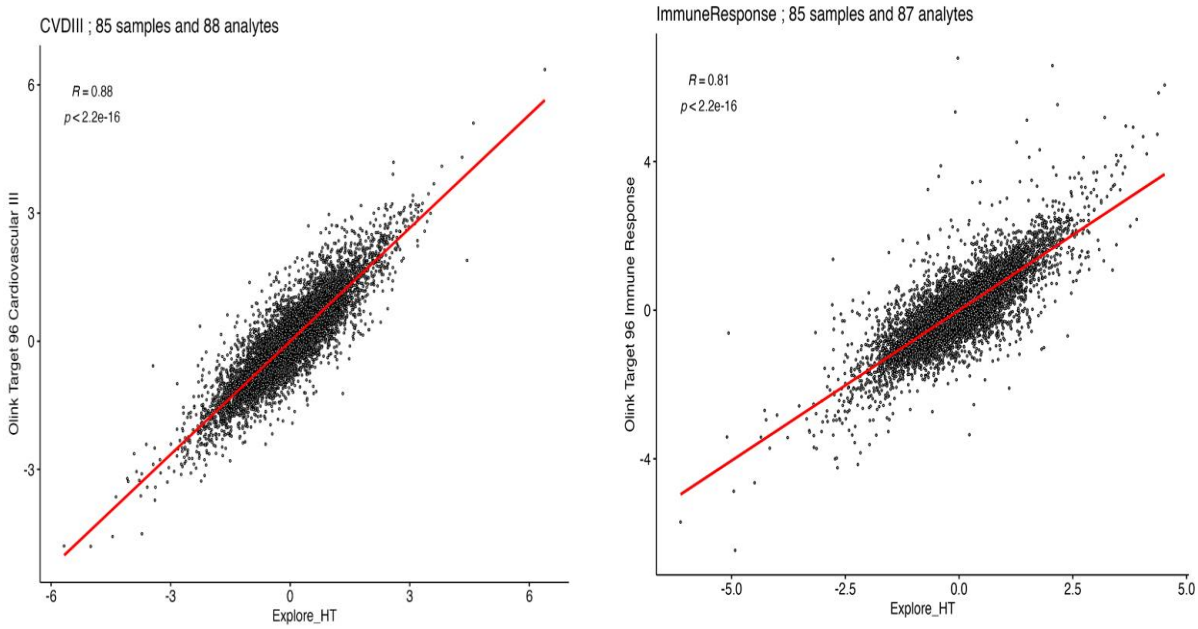

Figure 13. Scatter plot of data from Olink Explore HT and Olink Target 96 CVDIII or Immune Reponse panel present the linear relationship between two platforms.

The comparison of protein dynamic expression (z-scored NPX value) between Olink Explore HT and other 8 Olink Target 96 panels showed consistent pattern. See Figure 14 as representative graph. The protein expression dynamics and median z-score for 89 overlapping analytes were evaluated between Olink Explore HT and Target 96 Immuno-Oncology panel correlate for 101 plasma samples.

Figure 14. z-scored NPX for each overlapping analyte between Olink Explore HT and Olink Target 96 Immuno-Oncology Panel

4. Conclusion

The performance of Olink Explore HT has been tested and replicated in concordance sample data, and biological samples. The precision of Olink Explore HT is in-line with Olink Target 96 and Olink Explore 3072, while significantly expanding the protein library to expand the coverage of biological processes. Olink has strong concordance between products across the entire portfolio, with detectability accurately reflecting biology. Olink

Explore HT is confirmed as a robust and reliable product, delivering high-quality data to customers at an unprecedented scale.

HIMC has participated the Olink proficiency testing and was qualified as certified service provider for Olink Explore HT assays.