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Simplifying Progress

Development and Qualification of a Complex Potency ELISA

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Introduction

Programmed cell death protein 1 (PD-1) is an immune checkpoint that can be found on cells involved in regulating the immune system's response to self cells by down regulating the immune response and promoting self tolerance by suppressing T cell inflammatory activity. Therefore, PD-1 has an important role in preventing autoimmune diseases but can also prevent the immune system from killing cancer cells. PD-1, which can be found on T cells and some B cells, normally interacts with its two ligands PD-L1 and PD-L2, found on antigen-presenting cells and tumor cells. Many new therapies are targeting the PD-1 pathway to boost the immune response to cancer cells.

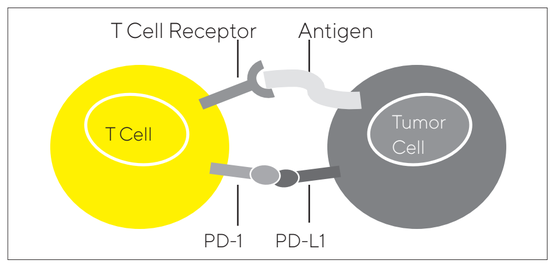


Figure 1: Immune checkpoints: PD-1 pathway . T Cells require two signals to become fully activated: 1. Antigen-specific interaction of T Cell Receptor (TCR) with peptide-MHC molecules. 2. Antigen non-specific co-stimulatory signal

Current assays used to measure the activity of anti-PD-1 or anti-PD-L1 antibodies rely on primary human T cells and measurement of functional endpoints such as cell proliferation, interferon gamma (IFNγ) and interleukin-2 (IL-2) production. These assays can be highly variable due to their reliance on primary cells and complex assay protocols. One method to demonstrate the activity of anti PD-1 molecules using a functionally relevant but less variable method is using a potency ELISA.

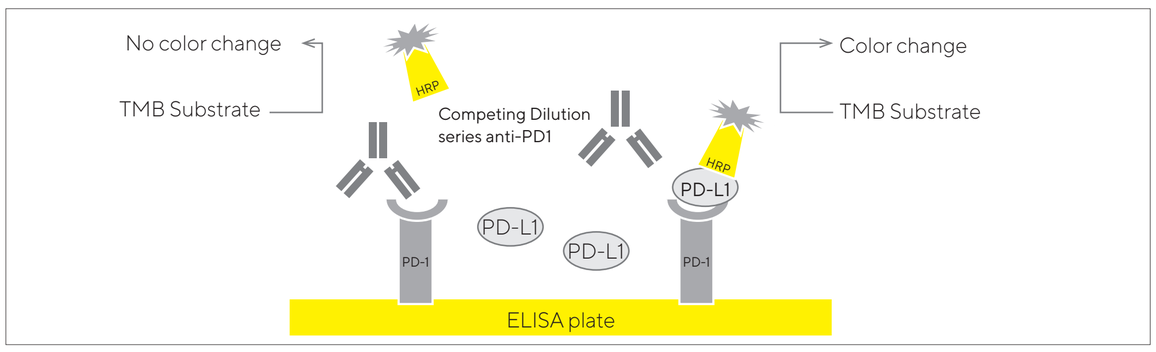


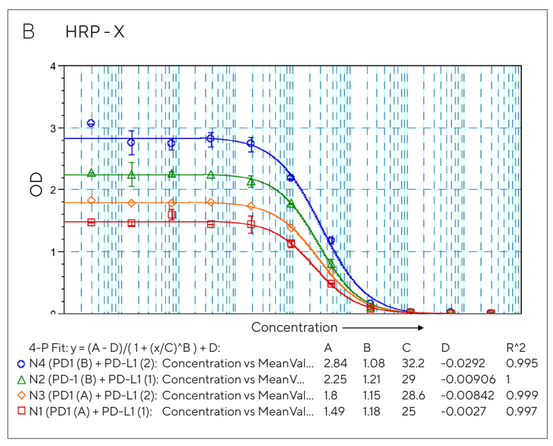
Figure 2: Schematic diagram of the mode of action reflective potency ELISA

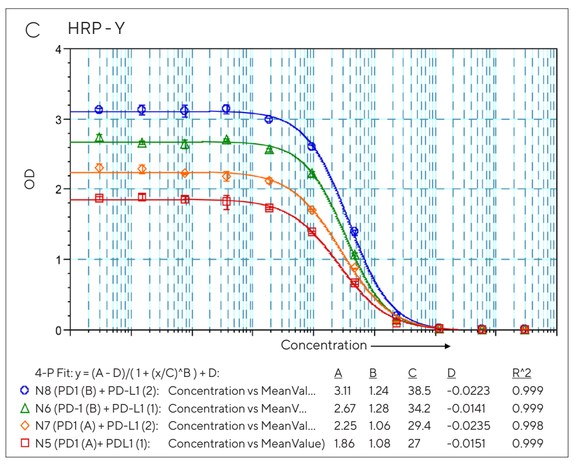
Development of the Potency ELISA

Using Modde Pro software a full factorial design of experiment approach was used to begin development of the Potency ELISA.

A

Exp Name	Run Order	PD-1 (Concentration)	PD-L1 (Concentration)	HRP (dilution Factor)
N1	3	A	1	X
N2	2	B	1	X
N3	7	A	2	X
N4	1	B	2	X
N5	4	A	1	Y
N6	5	B	1	Y
N7	6	A	2	Y
N8	8	B	2	Y
N9	11	C	3	Z
N10	9	C	3	Z
N11	10	C	3	Z





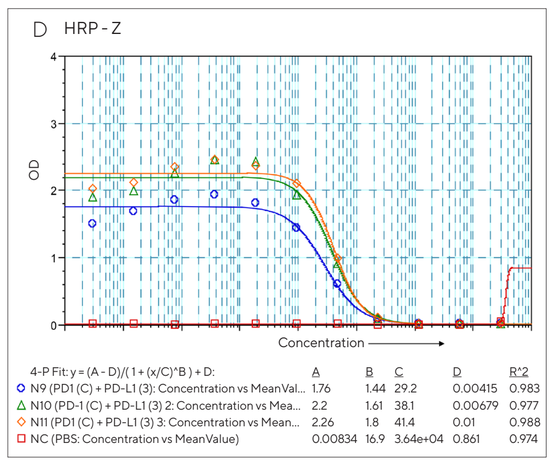
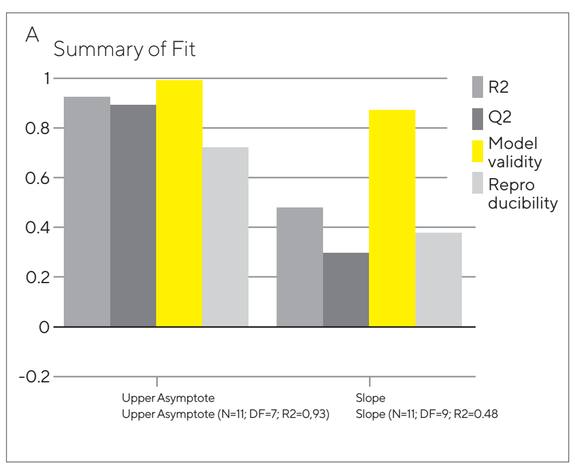


Figure 3. (A) DoE full factorial run order (11 runs anonymized). The factors assessed were as follows: three concentrations of PD-1 (A, B and C) are assessed along with three concentrations of PD-L1 (1, 2 and 3) and three concentrations of HRP (X, Y and Z). (B) Graphed results of Experiments N1 to N4 (C) Graphed results of experiments N5 to N8 (D) Graphed results of experiments N9 to N11.



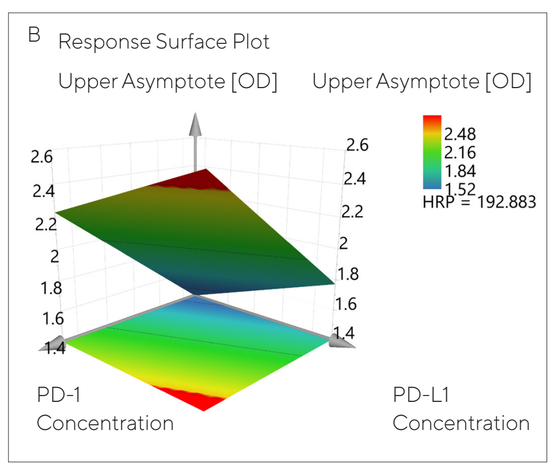
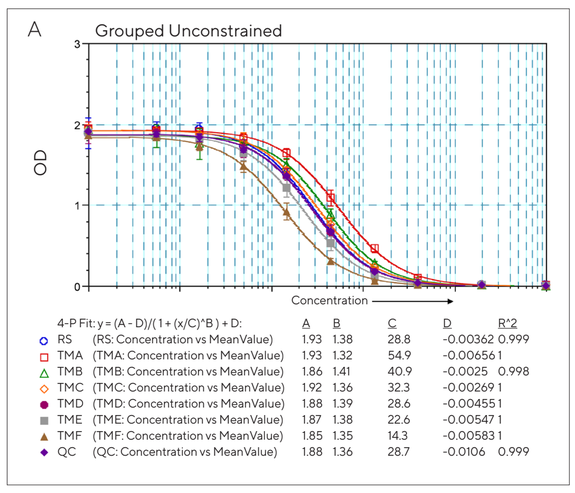


Figure 4. (A) Graph displaying the summary of fit for the Upper asymptote and Slope using the results generated in figure 3. The overall fit was much better using results generated for the Upper asymptote, therefore this was primarily used to assess the parameters. (B) Response contour surface plot generated using the optimizer setting. HRP set at optimized point, as determined by the software. Increasing PD-1 concentration displayed on the X-axis, increasing PD-L1 concentration on the Y-axis and the Upper asymptote results on the Z-axis.



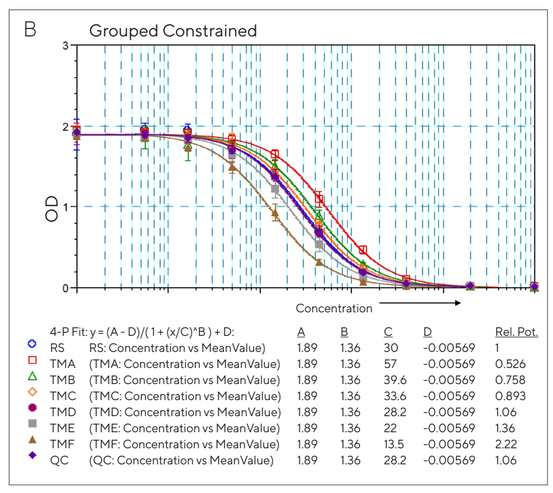
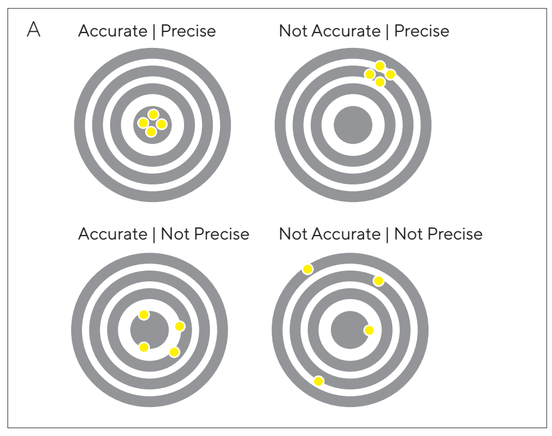


Figure 5: Accuracy assessments performed with optimized dilution series to better capture the profile of the curve. This was an assessment of the final methodology prior to qualification. The accuracy, relative confidence intervals, range and R2 values were all within an acceptable range.

A potency ELISA with the capabilities to assess up to 6 test material along with a reference standard and QC was developed for assessing anti-PD1 molecules.

Qualification of the Potency ELISA

ICHQ2 states the following characteristics should be evaluated when validating an analytical method. At Biooutsource we use these guidelines as the basis for qualifying our off-the-shelf assays for biosimilar comparability studies. We assess a wide range of concentrations in our assays which allows us to support clients from clone selection, through the definition of Critical Quality Attributes from an innovator population, right to finished product comparability studies.



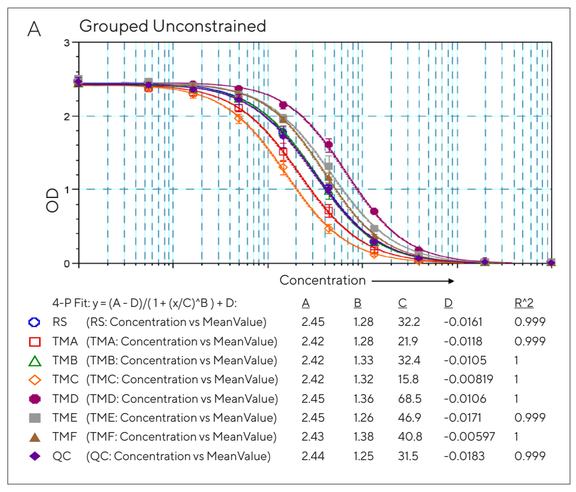
B

Concentration	Minimum number of assessments	Purpose
50%	3	Accuray, Range, Linearity & Intermediate Precision
70%	2	Accuracy, range and Linearity
80%	2	Accuracy, range and Linearity
100%	3	Accuray, Range, Linearity & Intermediate Precision
100%	3	Repeatability
100%	1	Specificity
125%	2	Accuracy, range and Linearity
143%	2	Accuracy, range and Linearity
200%	3	Accuray, Range, Linearity & Intermediate Precision

Figure 6: (A) Schematic of Accuracy and precision. (B) Qualification assessments required.

Results

Each assay performed could assess up to 6 test material along with a Reference Standard, and QC material.



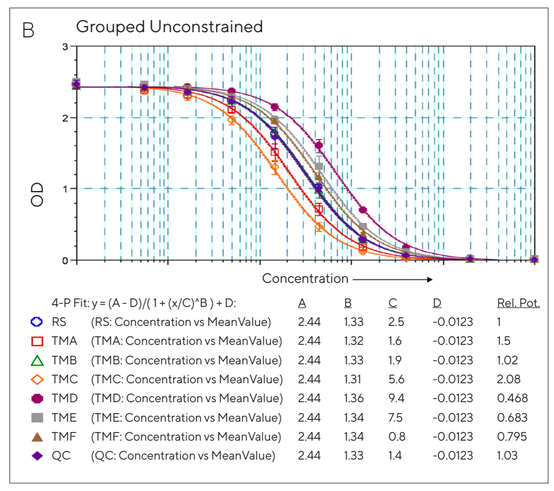
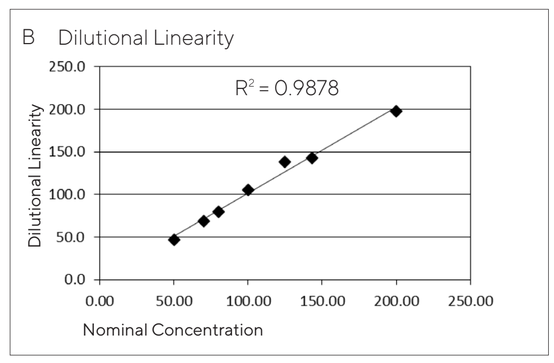


Figure 7: (A) Unconstrained Graph demonstrating the accuracy of samples at 150%, 100%, 200%, 50%, 70%, and 80% along with a QC (100%). (B) Constrained Graph.

A

Nominal Concentration	Result	% Accuracy
50	49.5	99.0
50	46.7	93.4
50	45.9	91.8
70	71.9	102.7
70	68.3	97.6
70	65.7	93.9
80	84.2	105.3
80	79.5	99.4
80	75.9	94.9
100	110.0	110.0
100	101.7	101.7
100	97.1	97.1
100	108.4	108.4
100	114.5	114.5
100	115.4	115.4
125	141.6	113.3
125	135.2	108.2
143	150.3	105.1
143	135.8	95.0
200	208.8	104.4
200	203.8	101.9
200	181.9	91.0
Specificity	0.0	N|A



Intermediate Precision (50%)	Intermediate Precision (100%)	Intermediate Precision (200%)
4%CV	6%CV	7%CV

Figure 8: (A) Tabulated results displaying all accuracy assessments performed during the qualification of the potency ELISA. (B) Graphed Dilutional linearity of the assay across the range of 50 to 200%. (C) Intermediate precision results were generated at 50%, 100% and 200%. The accuracy, intermediate precision, range and R2 values were all within an acceptable range.

Conclusion

Using a DoE approach, Sartorius Stedim Biooutsource have been able to apply a systematic approach to develop a complex ELISA. Following the principles set out in the ICHQ2 guidelines, we have demonstrated the potency ELISA to be an accurate and precise assay suitable for the characterization of multiple anti PD-1 molecules.