Highly sensitive analysis using EVOSEP-LC-MS/MS assay for targeted PD-L1 and PD-1 expression level for predicting response to immune checkpoint inhibitors

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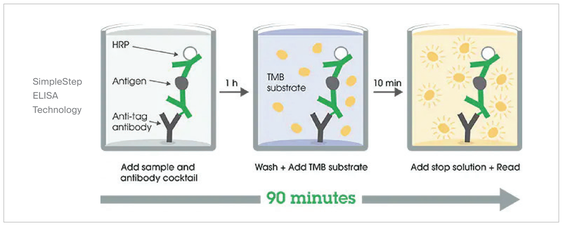
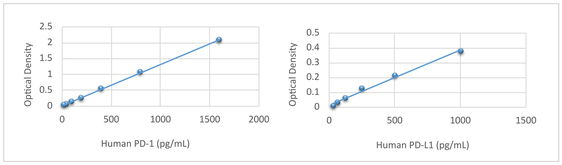
The use of monoclonal therapeutic antibodies targeting PD-1/PD-L1 axis completely changed anticancer treatment strategies. PD-L1 and PD1 immunoassays have been traditionally used for predicting clinical responses to immune checkpoint inhibitors (ICIs). However, PD-L1 and PD-1 as biomarkers show limitations. Among them, there is no consensus about the antibodies used to determine PD-L1 and PD-1 expression levels leading to uncertainty about the universal clinical value of these biomarkers across tumor types. In this work, we set to test a robust method to determine PD-L1 and PD-1 expression levels as compared to routinely used immunoassay at the analytical level and then use the Overall Response Rate (ORR) to assess clinical utility.

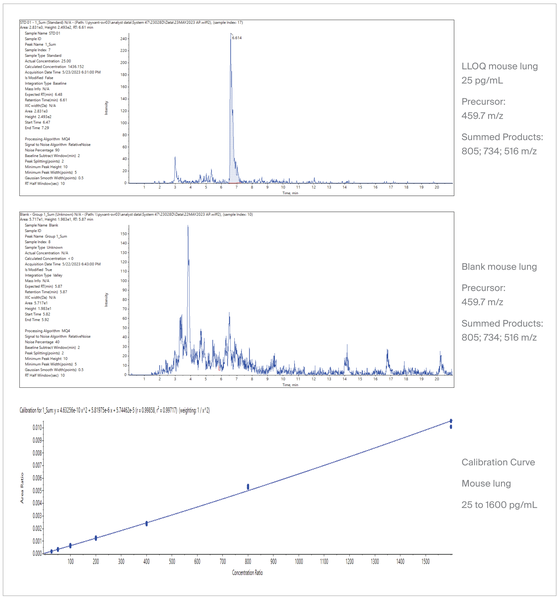
ELISA METHOD

Sandwich enzyme-linked immunosorbent assays (ELISA) were performed to detect and quantify PD-1 and PD-L1 in non-small cell lung cancer (NSCLC) tissue lysates. The following kits were used: Human PD-1 ELISA Kit (ab252360) and Human Programed Death Ligand-1 (PD-L1) ELISA Kit (MBS9311868). The assays tested each sample in triplicate in a series of dilutions. The signal intensity was measured using a microplate reader at the appropriate wavelength. The concentrations of the target analyte in the samples were determined by comparing their signal intensities to a standard curve generated from known concentrations of the analyte.





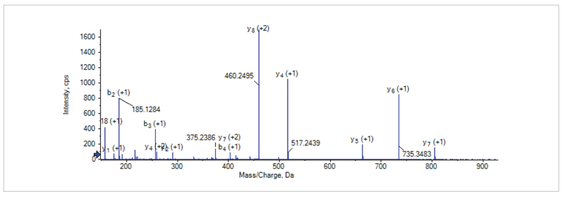
LCMS DATA



MS DEVELOPMENT

Peptide mapping utilizing IDA data acquired with a SCIEX 7600 Zeno TOF system was performed to determine the best surrogate peptide to monitor for quantitation. For PD1, two peptides were selected based on sensitivity and uniqueness: LAAFPEDR and DFHMSVVR. Ultimately, DFHMSVVR was not used for quantitation due to the potential to oxidize the methionine amino acid. For PD-L1, FTVTVPK was selected and used for quantitation.

For analysis, an MRM-HR experiment was used, and the top three product ions were summed for quantitation.

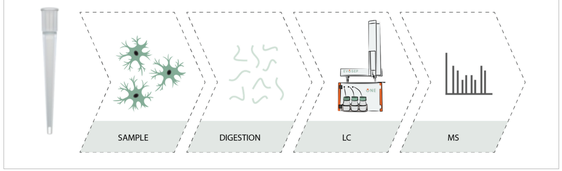


MSMS: LAAFPEDR with Y and B ions identified

EVOSEP OVERVIEW AND METHODOLOGY

The EVOSEP ONE is a high-throughput liquid chromatographic system that uses individual disposable trap columns built into a pipette tip format. EVOSEP ONE is designed to use preconfigured LC gradient methods utilizing µL/min flow rates.

For this work, a 60-sample-a-day method utilizing an 8 cm x 100 µm column with a 1.9 µm particle size was used (EVOSEP P/N EV1064)



PRE-EVOSEP ONE DIGESTION METHOD

Tissue samples (~ 25 mg) were homogenized in T-CEP at a ratio of 1:10 using a Geno/Grinder. After homogenization, the samples were treated as follows:

- 25 µL of homogenate is diluted with 80 µL of 100 mM Ammonium bicarbonate buffer and 10 µL dithiothreitol and incubated at 50°C for 60 mins with agitation.
- After cooling to RT, 10 µL of iodoacetamide is added and incubated in the dark at 22°C for 30 mins with agitation.
- After incubation, 10 µL of 4 mg/mL trypsin is added and incubated at 37°C for 5 hrs. with agitation.
- The reaction is stopped by adding 10 µL of 50/50 water/formic acid.
- LAAFPEDR^ is added as an internal standard for PD-1
- 50 µL of sample is then processed following a standard EVOTIP protocol

ELISA CALIBRATOR A/P RESULTS

REPLICATES	25 PG/ML	50 PG/ML	100 PG/ML	200 PG/ML	400 PG/ML	800 PG/ML	1600 PG/ML
1	16.69	48.23	105.92	181.31	424.38	808.23	1595.15
2	10.54	49.77	112.84	212.08	430.53	732.84	1355.92
MEAN	13.61	49.00	109.38	196.69	427.46	770.53	1475.53
PERCENT CV	31.96	2.22	4.48	11.06	1.02	6.92	11.46
ACCURACY	54%	98%	109%	98%	107%	96%	92%

LC/MS CALIBRATOR AND QC RESULTS

REPLICATE	LOW 75 PG/ML	MEDIUM 335 PG/ML	HIGH 1350 PG/ML
1	62.6	342	1510
2	64.1	343	1560
3	63.7	334	1469
4	60.7	341	1560
5	65.8	323	1494
6	62.6	330	1601
MEAN	63.2	335	1532
PERCENT CV	2.68	2.35	3.23
ACCURACY	84.3%	95.9%	113.1%

REPLICATES	25 PG/ML	50 PG/ML	100 PG/ML	200 PG/ML	400 PG/ML	800 PG/ML	1600 PG/ML
1	25.2	49.9	105	206	400	839	1534
2	25.2	48.5	95.0	190	379	858	1599
MEAN	25.2	49.2	100	198	389	849	1567
PERCENT CV	0.05	2.0	7.3	5.6	3.8	1.6	2.9
ACCURACY	100.9%	98.4%	100.2%	99.1%	97.3%	106.1%	97.9%

ASSAY COMPARISON

- Equivalent sensitivity established between EVOSEP LC-MS/MS and ELISA methods with slightly greater precision for the LC-MS/MS
- The cost to process samples with EVOSEP is about half the cost of ELISA
- Instrument run time is greater with the EVOSEP LC-MS/MS

NEXT STEPS

- Compare LC-MS/MS and other ELISA methods for sensitivity, specificity, and throughput
- Determine if the LC-MS/MS method is adaptable to other matrices (e.g., plasma, whole blood, other tissues)

