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Expansion of Human Immuno-Oncology Assays on a Multiplexed Electrochemiluminescence Platform

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PURPOSE

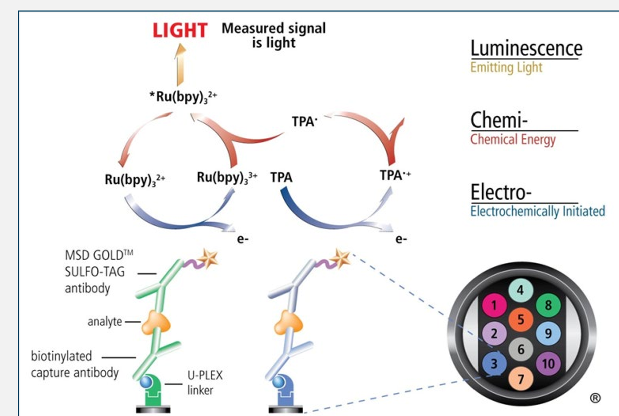
Novel developments in immuno-oncology research have driven an increased demand for the sensitive measurement of biomarkers associated with cancer, immune responses, and targets of therapeutic drugs. The levels of these biomarkers are frequently altered in samples from cancer patients and can be evaluated by measuring their concentrations in blood and tissue lysate samples. We had previously developed and launched 27 immuno-oncology assays targeting both traditional and emergent cancer biomarkers. We now report expansion of this group with the introduction of 24 additional assays. The assays are available on the U-PLEX platform and can be multiplexed with 80 other U-PLEX assays. The combined group of 131 assays allows for flexible and simultaneous measurements of immuno-oncology, inflammation, and metabolic analytes.

METHODS

Electrochemiluminescence Technology

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.

- Minimal background, combined with strong response to analyte, yields high signal-to-noise ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.



U-PLEX® Immuno-Oncology Protocol

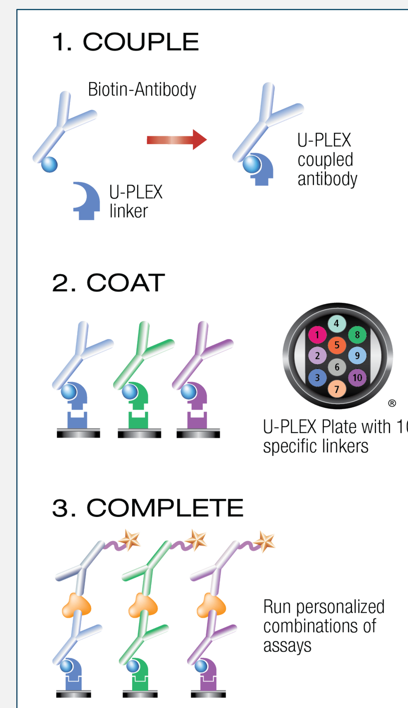
The U-PLEX assay platform uses 10 unique linkers that specifically bind to individual spots, enabling simple and flexible creation of multiplex immunoassays.

Couple and Coat the U-PLEX Plate:

- Add 200 µL of the biotinylated capture antibody to 300 µL of the assigned linker. Vortex. Incubate for 30 minutes.
- Add 200 µL of Stop Solution and vortex. Incubate for 30 minutes.
- Combine each U-PLEX-coupled antibody solution into a single tube and vortex. Add 50 µL of multiplex coating solution to each well.
- Incubate with shaking for 1 hour then wash the plate.

Complete the Assay:

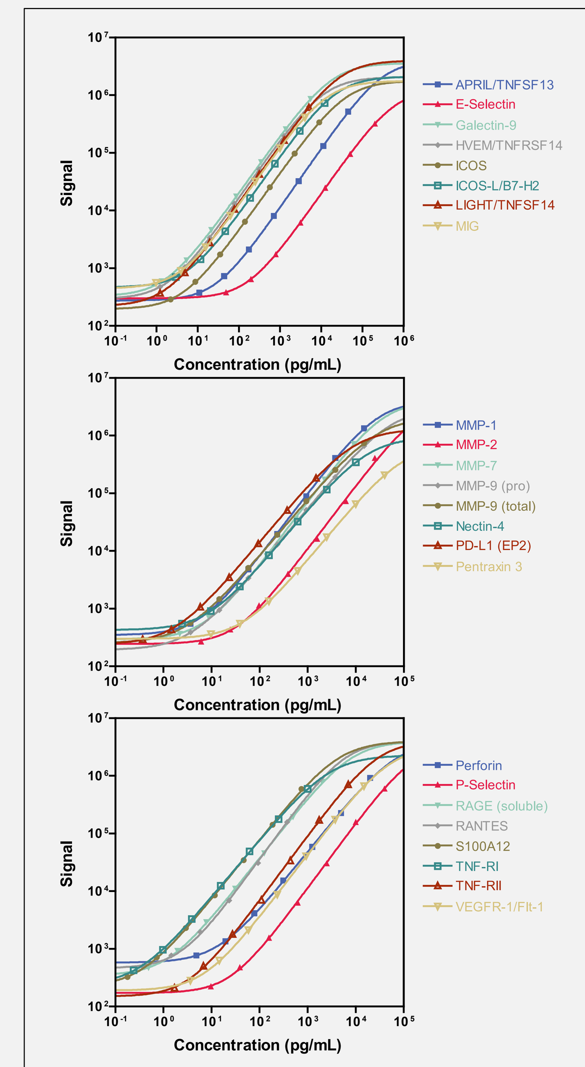
- Add 50 µL of sample, calibrator, or control to each well.
- Incubate the plate for 2 hours, then wash the plate.
- Add 50 µL of detection antibody solution to each well.
- Incubate the plate for 1 hour, then wash the plate.
- Add 150 µL of MSD® Read Buffer to each well and read the plate.



RESULTS

Assay Characteristics

Calibrator curves, lower limit of detection (LLOD), and upper limit of detection (ULOD) for 24 new human immuno-oncology assays are shown below. LLODs were calculated from 3 runs each with >20 blank wells. Control samples for each assay showed expected precision and accuracy, with intra-run CVs less than 10%, inter-run CVs less than 25%, and recoveries largely within 70-130% of target concentrations (data not shown).



Analyte	LLOD-ULOD (pg/mL)
APRIL/TNFSF13	7.47 - 45,000
E-Selectin	45 - 200,000
Galectin-9	0.41 - 5,500
HVEM/TNFRSF14	0.53 - 5,000
ICOS	1.78 - 9,000
ICOSL/B7-H2	0.98 - 12,000
LIGHT/TNFSF14	0.58 - 5,000
MIG	0.73 - 4,000
MMP-1	1.35 - 15,000
MMP-2	10.4 - 40,000
MMP-7	1.83 - 9,000
proMMP-9	0.88 - 15,000
MMP-9 (total)	1.61 - 10,000
Nectin-4	0.55 - 1,500
PD-L1 (epitope 1)	11.7 - 40,000
Pentraxin 3	1.92 - 20,000
Perforin	1.42 - 15,000
P-Selectin	10.5 - 40,000
RAGE (soluble)	0.26 - 2,000
RANTES	0.41 - 1,500
S100A12	0.1 - 750
TNF-RI	0.15 - 1,000
TNF-RII	1.6 - 7,000
VEGFR-1/Flt-1	2.69 - 15,000

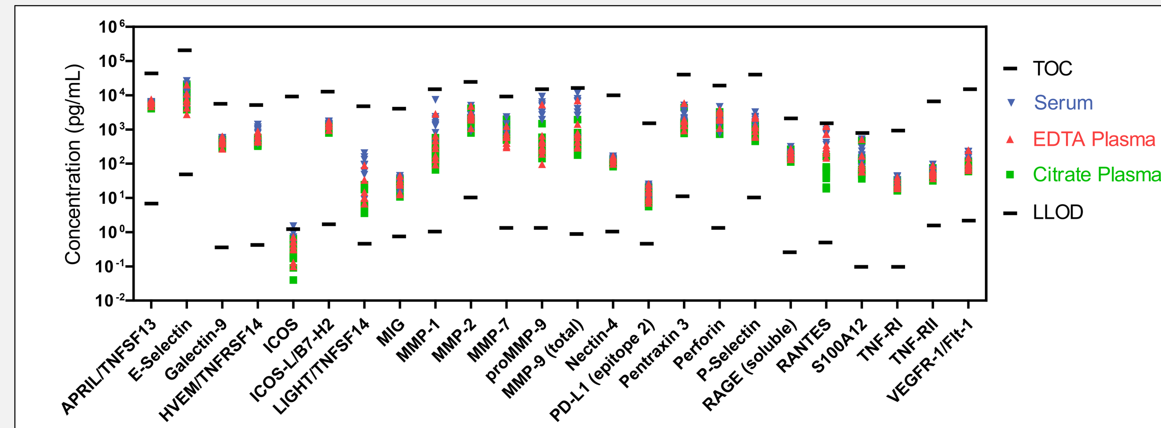
U-PLEX Biomarker Compatibility

Compatibility with existing human U-PLEX immuno-oncology assays was tested using dynamic range, sensitivity, sample detection, and non-specific binding as performance criteria. As a result, the U-PLEX Immuno-Oncology Group 1 (human) panel was expanded to 131 assays (see the table below) that can be multiplexed together.

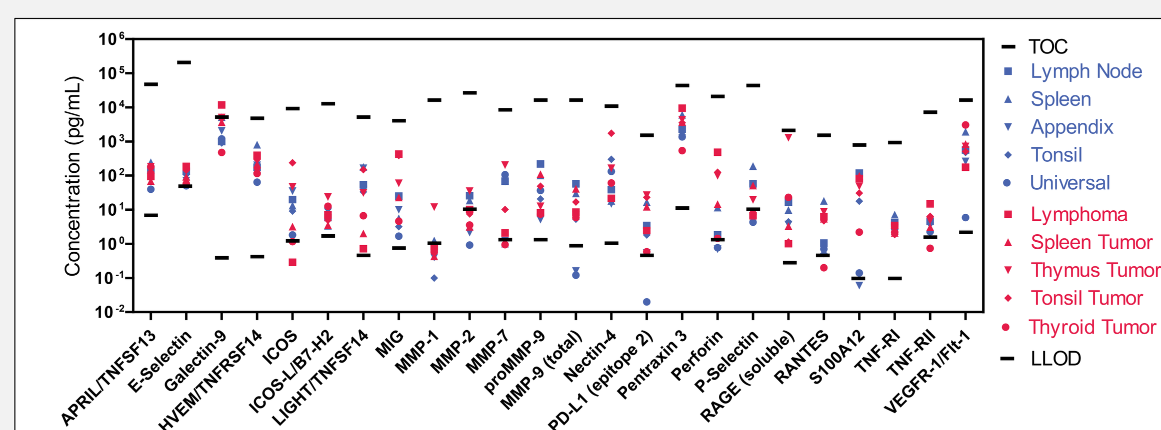
APRIL/TNFSF13	Galectin-9	IL-1α	IL-17E/IL-25	MIF	P-Selectin
BAFF-R/TNFRSF13C	G-CSF	IL-1ß	IL-17F	MIG	PYY (total)
BCMA/TNFRSF17	Ghrelin (active)	IL-1RA	IL-18	MIP-1α	RAGE (soluble)
CD20	GIP (active)	IL-2	IL-21	MIP-1ß	RANKL/TNFSF11
CD27	GIP (inactive)	IL-2Rα	IL-22	MIP-5	RANTES
CD276/B7-H3	GITR/TNFRSF18	IL-3	IL-23	MMP-1	S100A12
CD28	GITRL/TNFSF18	IL-4	IL-27	MMP-2	SDF-1α
CD40L (soluble)	GLP-1 (active)	IL-5	IL-29/IFN-λ1	MMP-7	Tie-2
C-Peptide	GLP-1 (inactive)	IL-6	IL-31	proMMP-9	TIGIT
CTACK	GM-CSF	IL-7	IL-33	MMP-9 (total)	TLR1
CTLA-4	gp130 (soluble)	IL-8	Insulin	Nectin-4	TNF-α
ENA-78	Granzyme A	IL-9	IP-10	OX40/TNFRSF4	TNF-RI
Eotaxin	Granzyme B	IL-10	I-TAC	PD1 (epitope 1)	TNF-RII
Eotaxin-2	GRO-α	IL-12/IL-23p40	LAG3	PD1 (epitope 2)	TNF-ß
Eotaxin-3	HAVCR2/TIM-3	IL-12p70	Leptin	PD-L1 (epitope 1)	TPO
EPO	HVEM/TNFRSF14	IL-13	LH	PD-L1 (epitope 1)	TRAIL
E-Selectin	I-309	IL-15	LIGHT/TNFSF14	PD-L2	TSLP
FGF (basic)	ICOS	IL-16	MCP-1	Pentraxin 3	VEGF-A
FGF-23	ICOSL/B7-H2	IL-17A	MCP-2	Perforin	VEGF-D
FLT3L	IFN-α2a	IL-17A/F	MCP-4	PlGF	VEGFR-1/Flt-1
Fractalkine	IFN-ß	IL-17C	M-CSF	PP	YKL-40
FSH	IFN-γ	IL-17D	MDC	Proinsulin	

Native Sample Testing

Immuno-oncology assays were evaluated for the ability to detect their respective analytes in human serum, EDTA plasma, and citrate plasma samples. Sample concentrations (pg/mL) were plotted with the top of curve (TOC) and LLOD for each analyte. Samples were diluted 4-fold except for ICOSL/B7-H2, MMP-2, proMMP-9, MMP-9 (total), P-Selectin, RANTES, S100A12, TNF-RI and TNF-RII assays where samples were diluted 100-fold. ICOS was not detected in human serum and plasma samples. All other analytes were detected irrespective of the type of matrix.

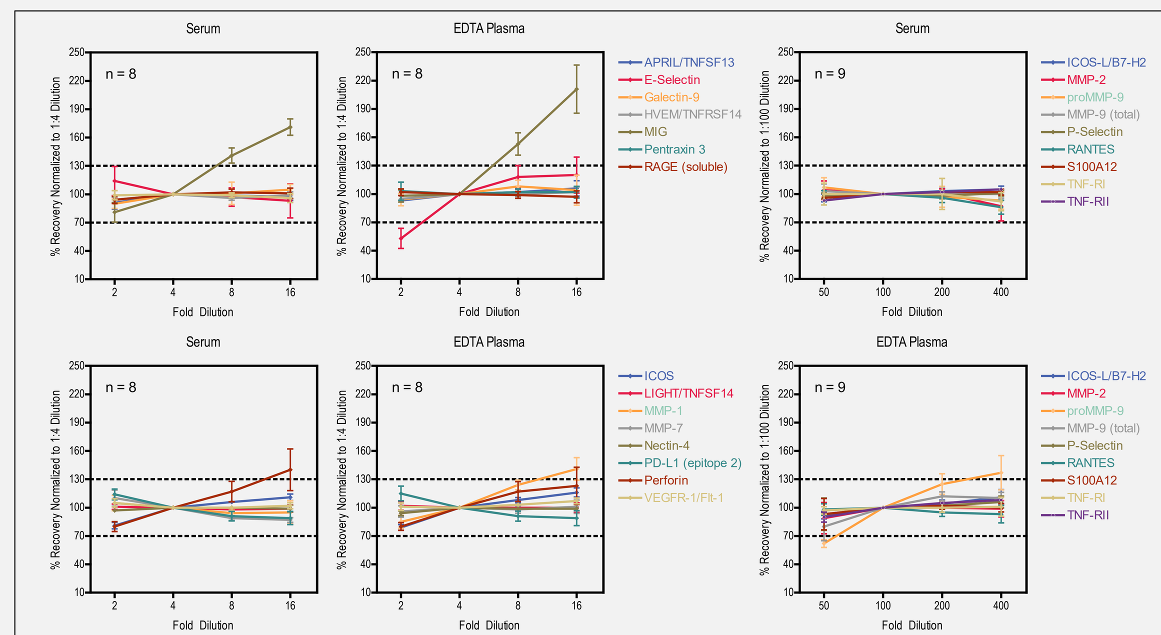


Human tissue lysate samples (6.25 µg) derived from different normal tissues (blue symbols) and tumor tissues (red symbols) were tested. Sample concentrations (pg/mL) were plotted with TOC and LLOD values for each analyte. All analytes were detected in most of the tissue lysate samples.



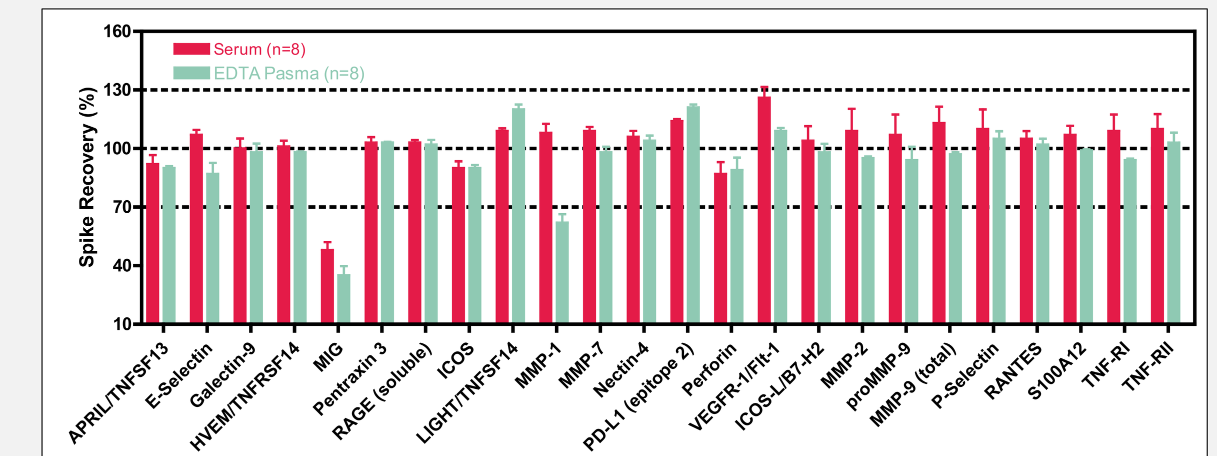
Dilution Linearity

Serum and EDTA plasma samples were spiked with calibrator and diluted 2, 4, 8, and 16-fold before testing. Sample concentrations were normalized to the 4-fold sample dilution. For ICOS-L/B7-H2, MMP-2, proMMP-9, MMP-9 (total), P-Selectin, RANTES, S100A12, TNF-RI and TNF-RII, unspiked samples were diluted 50, 100, 200 and 400-fold. Sample concentrations for these were normalized to the 100-fold sample dilution. Most analytes recovered within 70-130% in each type of sample. Recovery of MIG improved with the addition of 0.1% Triton X-100 (data not shown).



Spike Recovery

Normal human serum and EDTA plasma samples were spiked with calibrators at 3 levels (high, mid, and low). Spike recovery values for the three spike levels were averaged and plotted. Recovery of most analytes was within 70-130% in each sample type.



Assay Interference and Competition

Immuno-oncology assays were evaluated for interference and competition with therapeutic antibody drugs and homologous and/or related analytes. Assay interference was measured by comparing recovery of analyte in the presence of a wide concentration range of the potential interferent. Competition was measured by comparing human serum and plasma sample concentrations in singleplex and multiplex formats. Percent change from control was reported in the table below (% change >50% shaded red, <50% shaded yellow, <20% shaded green). Testing with therapeutic antibody drugs demonstrated that the PD1 (epitope 1) assay is more resistant to Nivolumab and Pembrolizumab than PD1 (epitope 2). Similarly, PD-L1 (epitope 2) is more resistant to Atezolizumab than PD-L1 (epitope 1).

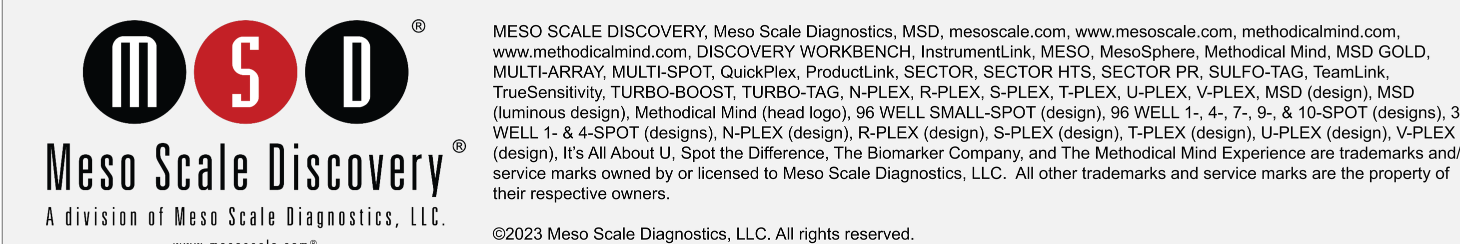
Assay	Interferent	Impact
APRIL/TNFSF13	BAFF-R/TNFRSF13C	—
	BCMA/TNFRSF17	—
BAFF-R/TNFRSF13C	APRIL/TNFSF13	—
	BAFF	—
	BCMA/TNFRSF17	—
	TACI/TNFRSF13B	—
BCMA/TNFSF17	APRIL/TNFSF13	—
	BAFF	—
CD28	CD80/B7-1	—
	CTLA-4	—
CTLA-4	Ipilimumab	-90%
	CD28	—
	CD80/B7-1	—
Galectin-9	HVACR2/TIM-3	—
Granzyme A	Perforin	—
	Prot. Inhibitors	—
GITR/TNFSRF18	GITRL/TNFSF18	—

Assay	Interferent	Impact
gp130 (soluble)	IL-6	—
HVEM/TNFRSF14	LIGHT/TNFSF14	—
HVACR2/TIM-3	Galectin-9	—
ICOS	ICOS-L/B7-H2	—
ICOS-L/B7-H2	ICOS	+20%
LIGHT/TNFSF14	HVEM/TNFRSF14	—
OX40/TNFRSF4	OX40L/TNFSF4	—
PD1 (epitope 1)	Nivolumab	-33%
	Pembrolizumab	-19%
	PD-L1	—
	PD-L2	—
PD1 (epitope 2)	Nivolumab	-99%
	Pembrolizumab	-98%
PD-L1 (epitope 1)	Atezolizumab	-91%
	PD1	—
	PD-L2	—

Assay	Interferent	Impact
PD-L1 (epitope 2)	PD-1	—
	Atezolizumab	-17%
PD-L2	PD1	—
	PD-L1	—
Perforin	Granzyme A	—
PlGF	VEGFR-1/Flt-1	—
RANKL/TNFSF11	Osteoprotegerin	—
TIGIT	CD155	—
TNF-α	TNF-RI	—
TNF-β	TNF-RI	—
	TNF-RII	—
TNF-RI	TNF-α	—
	TNF-β	—
TNF-RII	TNF-β	—
VEGF-A	VEGFR-1/Flt-1	—
VEGFR-1/Flt-1	PlGF	-27%

CONCLUSIONS

The U-PLEX immuno-oncology assay portfolio has expanded to 51 assays with the addition of twenty-four new human assays. U-PLEX immuno-oncology assays can be used in singleplex and multiplex formats and can be run in combination with 80 additional biomarker assays bringing the total number of compatible assays to 131. These assays enable researchers and drug developers to simultaneously measure immuno-oncology analytes along with cytokines, chemokines, and inflammatory markers.



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