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Development of a Flexible, Personalized Multiplexing Platform

C. Lu, P. Liu, C. Smith, E. Kukreti, J. Han, L. Dzantiev, B. Sun, J. Zhang, D. Stewart, M. Higgins, C. Frye, J. Suschak, J. Kenten, S. Kumar, E.N. Glezer, G.B. Sigal, P. Oberoi, J. Wilbur, and J.N. Wohlstadter

Meso Scale Discovery, Rockville, Maryland, USA

1 Abstract

Introduction: Creating multiplex assays utilizes complex procedures for conjugating biological reagents to beads, or advanced technologies to dispense nanoliters of fluid to arrays. The U-PLEX® assay platform enables the flexible creation of multiplex assays using MSD's MULTI-ARRAY® technology. The platform is designed to be used with readily available biotin-conjugated reagents including antibodies, peptides, proteins, and nucleic acids.

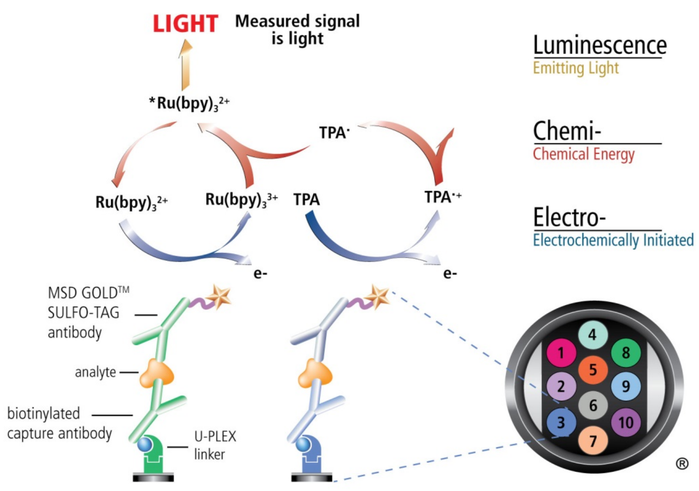
Materials and Methods: The U-PLEX platform consists of 10 unique U-PLEX linkers that specifically bind to 10 individual spots on a 96-well U-PLEX plate. The biotin-based capture coupling mechanism involves a two-step process: (1) a linker is bound to a biotinylated antibody and (2) the linker-coupled antibody is bound to the plate. The assay is completed using a simple workflow; the sample is added, a wash step performed, and the biomolecule is detected using a SULFO-TAG™ labeled detector. The platform was tested for assay specificity, stability, and reproducibility.

Results: Linkers are highly specific for their corresponding spots (< 0.02% non-specificity). The intra-plate CVs of antibody captured on the U-PLEX plate are ~4%. Inter-plate CVs are ~6%. Assays transferred to the platform exhibit good sensitivity, large dynamic range (4-5 logs), precision, and sample quantification. Serum, plasma, and PBMC samples run on the U-PLEX platform showed a high level of correlation (r^2 > 0.9) to the samples run on MSD's V-PLEX® assays.

Conclusions: The U-PLEX platform provides a flexible system for preparing custom multiplex bioassays. Performance outputs demonstrate that this platform is suitable for high quality sample measurements with existing and/or novel assays.

2 Methods

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



Luminescence
Emitting Light

Chemi-
Chemical Energy

Electro-
Electrochemically Initiated

Electrochemiluminescence Technology

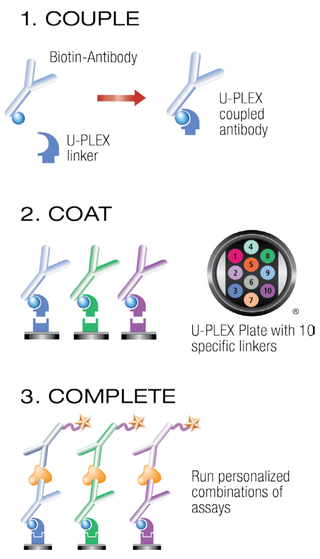
- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed 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.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

Typical U-PLEX Protocol

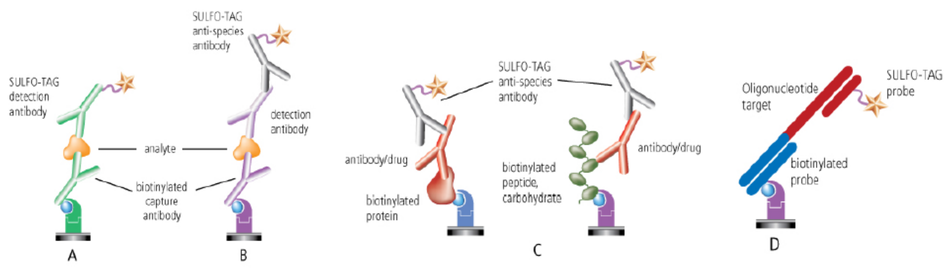
Create U-PLEX Coating Solution:
- Dilute each biotinylated antibody to 10 µg/mL in coating diluent.
- Couple each biotinylated antibody to a unique linker by adding 200 µL of the antibody to 300 µL of the assigned linker. Vortex. Incubate at room temperature (RT) for 30 minutes.
- Add 200 µL of Stop Solution. Vortex. Incubate at RT for 30 minutes.
- Combine 600 µL of each U-PLEX-coupled antibody solution into a single tube and vortex. Up to 10 U-PLEX-coupled antibodies can be pooled.

Coat U-PLEX Plate:
- Add 50 µL of multiplex coating solution to each well. Seal plate with an adhesive plate seal and incubate at RT with shaking for 1 hour or overnight.
- Wash plate 3 times with at least 150 µL/well of PBS-T or MSD® Wash Buffer.

Complete (Run Assay):
- Add 50 µL of sample, calibrator, or control to each well; shake for 1 hr and wash.
- Add 50 µL of detection antibody solution to each well; shake for 1 hr and wash.
- Add 150 µL of 2X Read Buffer T to each well and read.



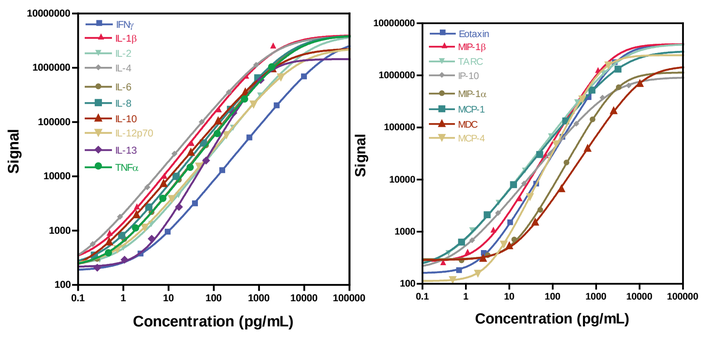
Assay Format



- In addition to building sandwich immunoassays, the U-PLEX platform can be used to measure proteins, antibodies, peptides, carbohydrates, polysaccharides, oligonucleotides, and nucleic acids (as illustrated above).

3 Performance of Assays on U-PLEX

Typical Standard Curves



- Assays performed on the U-PLEX assay platform typically show a 3-4 log dynamic range. This allows the user to simultaneously quantitate both normal and disease/stimulated samples with the same sample dilution.

Sensitivity

Assays	LLOD (pg/mL)									
	IFN-γ	IL-1β	IL-2	IL-4	IL-6	IL-8	IL-10	IL-12p70	IL-13	TNF-α
U-PLEX	1.13	0.07	0.33	0.04	0.23	0.13	0.10	0.29	1.92	0.16
MSD GOLD™ SS SA	12.2	0.10	0.22	0.07	0.28	0.13	0.13	0.18	1.10	0.16

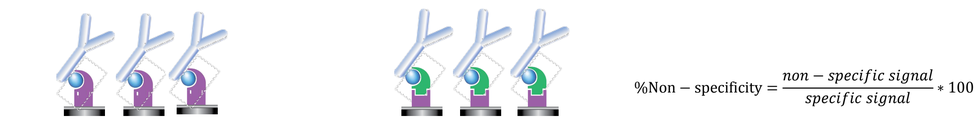
Assays	LLOD (pg/mL)							
	Eotaxin	IP-10	MCP-1	MCP-4	MDC	MIP-1α	MIP-1β	TARC
U-PLEX	1.18	0.26	0.19	3.51	4.75	2.54	0.95	0.16
MSD GOLD SS SA	1.61	0.30	0.19	9.55	0.83	5.74	0.47	0.20

- The sensitivity of assays on U-PLEX and MSD GOLD Small Spot Streptavidin plates were compared using the same antibodies. For each assay, the median lower limit of detection (LLOD) on U-PLEX was evaluated based on independent runs that were performed on at least 17 different plates.
- Our results showed that assays performed on both the U-PLEX and MSD GOLD Small Spot Streptavidin plates yielded similar results (LLODs).

Sample Quantitation

- Human serum and EDTA plasma samples were tested on both the U-PLEX and V-PLEX assays (using the same antibodies, calibrators and diluents). We found that there was a high correlation between the V-PLEX assays and other similar assays that were performed on the U-PLEX platform.

4 Specificity of Linkers

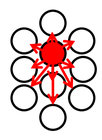


Specificity of the linkers was tested in two ways:
1. Individual U-PLEX capture materials were coated on single-spot plates and incubated with individual linker-coupled antibodies. The amount of antibodies on the plate surface was measured by SULFO-TAG conjugated Protein A/G. Linkers demonstrated high specificity for their corresponding capture materials with <0.02% non-specificity.

	Linker 1	Linker 2	Linker 3	Linker 4	Linker 5	Linker 6	Linker 7	Linker 8	Linker 9	Linker 10
Spot 1	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 2	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 3	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%
Spot 4	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 5	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 6	0.01%	0.00%	0.00%	0.01%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%
Spot 7	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%
Spot 8	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%	0.00%
Spot 9	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.00%
Spot 10	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%	100.00%

2. Individual linkers coupled with antibodies were tested on 10-spot U-PLEX plates. The amount of antibody on each spot was measured by SULFO-TAG conjugated Protein A/G. Non-specific binding from all combinations of linkers and spots was less than 0.03%.

	Linker 1	Linker 2	Linker 3	Linker 4	Linker 5	Linker 6	Linker 7	Linker 8	Linker 9	Linker 10
Spot 1	100.00%	0.02%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 2	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 3	0.00%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 4	0.01%	0.01%	0.00%	100.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%
Spot 5	0.00%	0.00%	0.00%	0.00%	100.00%	0.01%	0.00%	0.00%	0.00%	0.00%
Spot 6	0.00%	0.00%	0.00%	0.03%	0.00%	100.00%	0.00%	0.00%	0.00%	0.00%
Spot 7	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	100.00%	0.00%	0.00%	0.00%
Spot 8	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%	100.00%	0.01%	0.00%
Spot 9	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	0.00%	100.00%	0.01%
Spot 10	0.00%	0.00%	0.00%	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	100.00%

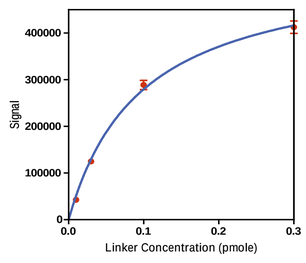


In this illustration, antibody-coupled linker 5 was incubated with a 10-spot U-PLEX plate. The non-specific interactions between linker 5 and all spots were tested.

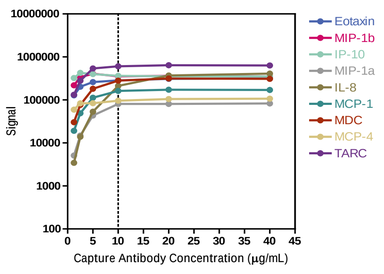
5 Reproducibility

Binding Capacity

The binding capacity of U-PLEX plates was measured by titrating linkers. The linkers were coupled with a biotinylated antibody and measured by SULFO-TAG conjugated protein A/G. A typical titration curve is shown below. The recommended linker concentration that should be used on a U-PLEX plate is 0.1 pmole per well. The table below shows that inter-plate %CVs from 14 plates at binding capacity was less than 10%.

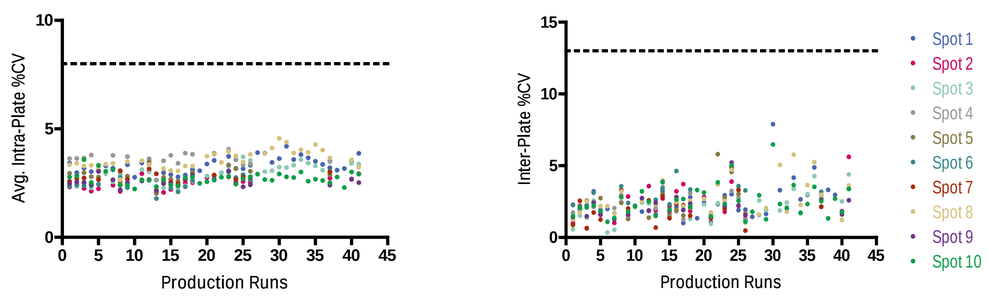


Linker Conc (pmole)	Inter-plate %CV									
	Spot 1	Spot 2	Spot 3	Spot 4	Spot 5	Spot 6	Spot 7	Spot 8	Spot 9	Spot 10
0.3	2.8	13.1	3.8	4.7	4.5	3.3	5.8	5.5	9.3	6.0
0.1	3.3	8.7	4.8	5.6	5.5	4.6	6.2	5.7	7.8	6.6
0.03	5.6	5.0	5.5	6.0	7.2	5.9	6.7	5.7	7.8	6.7
0.01	4.3	4.6	3.9	4.6	5.6	4.9	4.6	5.6	5.4	4.7



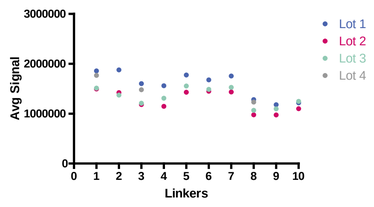
- To measure the amount of capture antibody required for coating a U-PLEX plate, biotinylated capture antibody titration was performed with 0.1 pmole of linkers. Once the antibody was immobilized to the plate through the U-PLEX linkers, the amount of antibody on the surface was determined using functional assays.
- Over 30 functional assays were tested (via step-wise protocol with plate washing). Representative data is shown on the left.
- The amount of biotinylated capture antibody needed for coating a plate is 10 µg/mL. At this concentration, less than 2 µg of antibody is used per 96-well plate.

Plate Reproducibility

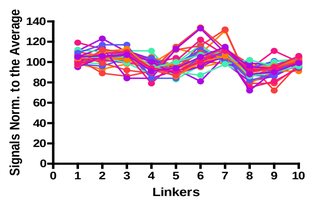


- Plate reproducibility was measured by running whole plates using linker-coupled antibody at binding capacity concentration. Once the antibody was immobilized by U-PLEX linkers, the amount of antibody remaining on the surface was determined using SULFO-TAG conjugated Protein A/G.
- The mean signal and CV were calculated for each plate (intra-plate %CV) and across plates (inter-plate %CV, 6 plates from each production run).
- 41 production runs of plates were tested for reproducibility. Intra-plate %CVs were below 5%, and inter-plate %CVs were below 8%.

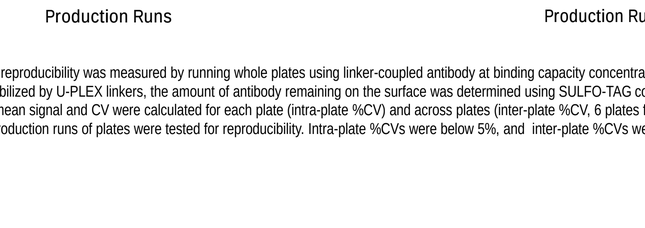
Linker Reproducibility



- Four lots of linkers were tested for lot-to-lot reproducibility. In each test, the linkers were coupled with a biotinylated antibody and measured using SULFO-TAG conjugated Protein A/G. The inter-lot %CV was less than 18% for all linkers.



- Thirty nine functional assays were tested on all 10 linkers. The assay signals on each linker were within 20% of the average signals.
- Representative data of functional assay signals are shown below.



6 Conclusion

U-PLEX is an assay development tool that enables researchers to create their own multiplex panels for any combination of analytes. The protocol is simple and easy: using a 10-spot U-PLEX plate and 10 unique linkers, a multiplex panel can be created in just 2 hours. The U-PLEX platform demonstrates excellent specificity and reproducibility, and is suitable for high quality sample measurements.

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