Immunogenicity Applications Using Electrochemiluminescence

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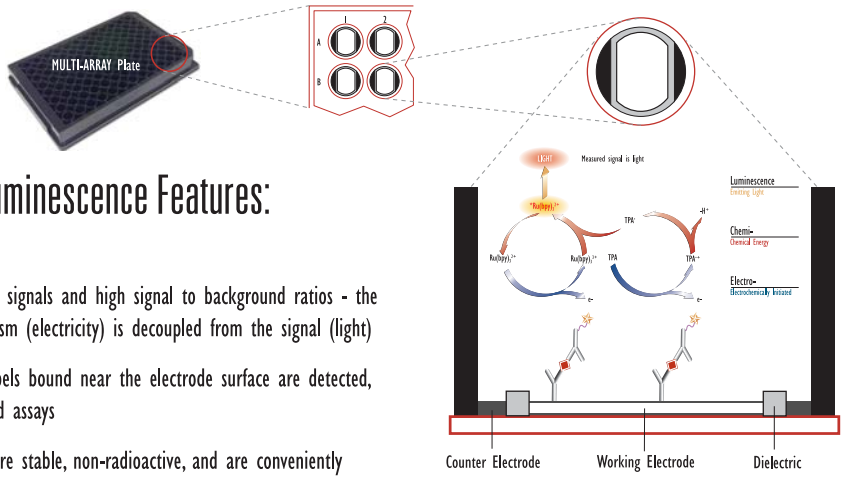
Immunogenicity testing is a crucial part of biopharmaceutical development. More stringent recommendations regarding immunogenicity assay performance necessitates the development of more robust and tolerant assays. MSD assays exhibit excellent sensitivity, precision, free drug tolerance, and minimal matrix effects. In addition, MSD assays are capable of finding low affinity antibodies during initial screens, and have a large linear range that reduces the number of required sample dilutions. Biopharmaceutical researchers can build assays for many drug types using MSD technology, including antibodies, humanized antibodies, proteins, and peptides with reagents designed to provide a variety of flexible assay formats and facilitate rapid assay development.

MSD Technology

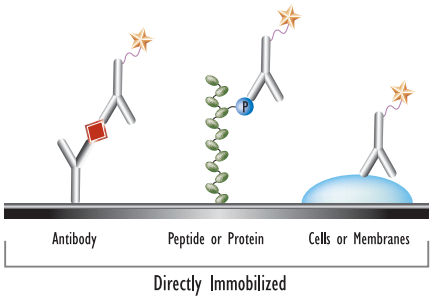
MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



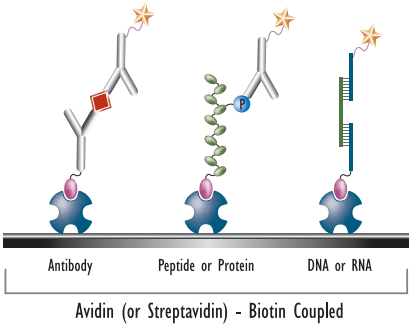
Electrochemiluminescence Features:

- Minimal background signals and high signal to background ratios - the stimulation mechanism (electricity) is decoupled from the signal (light)
- Proximity - only labels bound near the electrode surface are detected, enabling non-washed assays
- Flexibility - labels are stable, non-radioactive, and are conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminating problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity

Surface Chemistry



Directly Immobilized



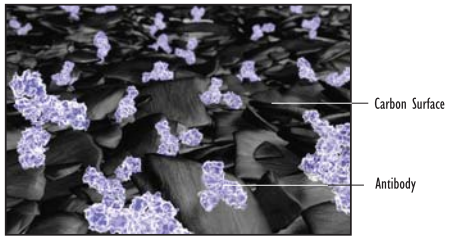
Avidin (or Streptavidin) - Biotin Coupled

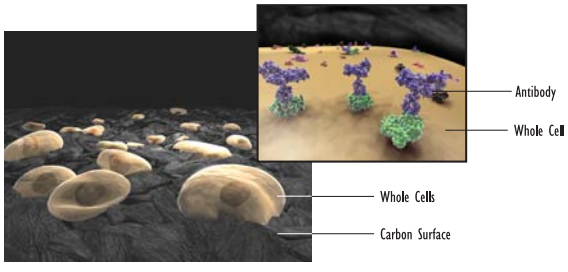
Immobilization on Uncoated Surfaces

- Capture Antibodies
- Immuno-Dot Blot Assays (Western Replacement)
- Receptor-Ligand Assays

Precoated Surfaces

- Avidin or Streptavidin
- Glutathione
- Antibodies





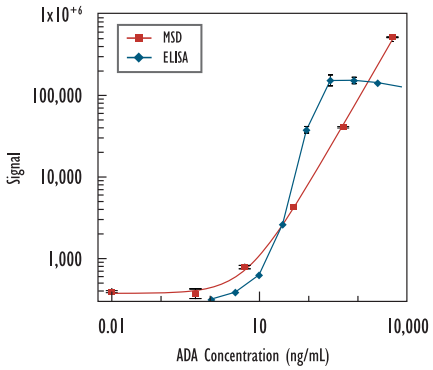
Immunogenicity

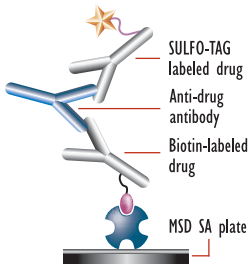
Immunogenicity testing is a crucial part of biopharmaceutical development. More stringent recommendations regarding immunogenicity assay performance necessitates the development of more robust and tolerant assays.
MSD assays exhibit excellent:

- Sensitivity
- Precision
- Free drug tolerance
- Insensitivity to matrix effects
- Detection sensitivity for low affinity antibodies, even in intial screens
- Dynamic Range - the large linear range reduces the number of required sample dilutions

Build assays for many drug types using MSD technology, including antibodies, humanized antibodies, proteins, and peptides with reagents designed to provide a variety of flexible assay formats and facilitate rapid assay development. Comparisons of MSD immunogenicity assays to the traditional ELISA format are featured below, using both a bridging assay format and direct immobilization format.

Bridging Immunogenicity Assay: ELISA Comparison





MSD assay shows comparable sensitivity to ELISA, with a larger dynamic range and a simple homogenous incubation.

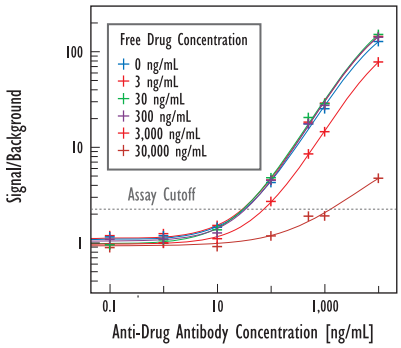
	ELISA	MSD
Better Free Drug Tolerance	Poor	Excellent
Detection of Low Affinity Antibodies	No	Yes
Fewer Washes	3-4	1
High-Throughput	Good	High
Direct Conjugation of Stable Label	Yes	Yes
Improved Sensitivity	100s ng/mL	10s ng/mL
Increased Dynamic Range	1-2 logs	3-4 logs
Reduced Sample Volume	25-100 µL	5-25 µL
Higher Binding Capacity		10X More

MSD Bridging Assay Protocol

1. Combine biotin-drug, sTAG-drug and sample in polypropylene plate and incubate 1 hour to overnight.
2. Transfer solution to pre-blocked standard streptavidin MSD plate. Incubate for 1 hour.
3. Wash assay plate; add Read Buffer T; read plate on SECTOR™ instrument.

Immunogenicity (continued)

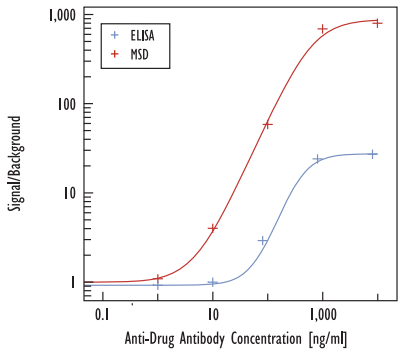
MSD Bridging Immunogenicity Assay in the Presence of Free Drug

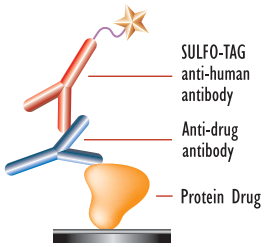


An example of an bridging immunogenicity assay is shown with different levels of free drug added to the sample (sample matrix was neat human serum).

- LOQ 30 ng/mL
- No effect on assay for free drug concentrations up to 300 ng/mL
- Assay can tolerate up to 3 µg/mL free drug at 100 ng/mL of anti-drug antibody

Direct Immunogenicity Assay for Protein Drugs





	ELISA	MSD
Better Free Drug Tolerance	Poor	Good
Detection of Low Affinity Antibodies	No	Maybe
Fewer Washes	3-5	2-3
High-Throughput	Good	Good
Direct Conjugation of Stable Label	No	No
Improved Sensitivity	100s ng/mL	10s ng/mL
Increased Dynamic Range	1-2 logs	3-4.5 logs
Reduction in Reagent Consumption		2-10 fold
Higher Binding Capacity		10X More

Neat human serum was used as the sample matrix. The top of the curve is about 1 µg/mL for both formats, but the MSD format is 40X more sensitive.

Reference: Moxness, M., Tatarewicz, S., Weeraratne, D., Murakami, N., Wullner, D., Mytych, D., Jawa, V., Koren, E., Swanson, S.J. (2005) **Immunogenicity Testing by Electrochemiluminescent Detection for Antibodies Directed against Therapeutic Human Monoclonal Antibodies.** Clinical Chemistry. 51: 1983-1985.

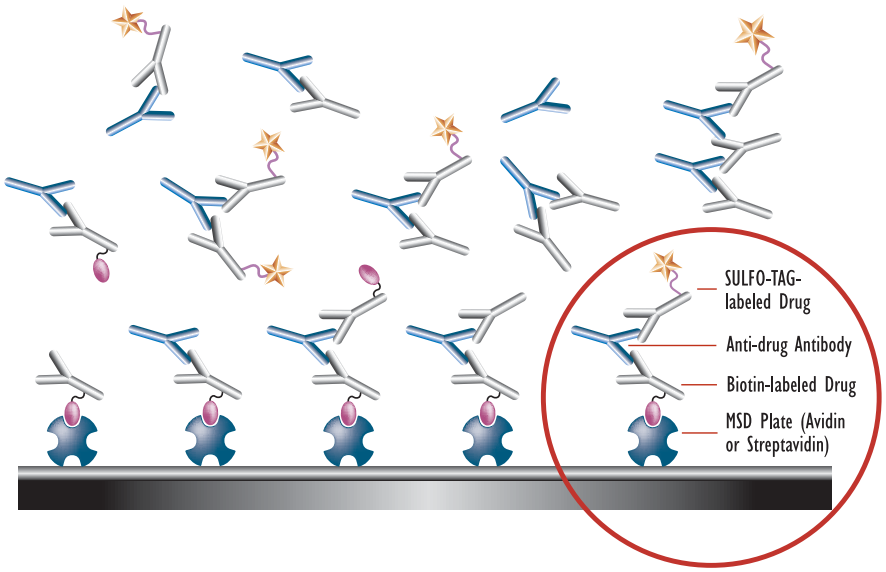
MSD Sandwich Immunogenicity Assay Protocol

1. Coat plate with drug at 0.05 to 5 pmole per well and incubate for 1 hour to overnight.
2. Block with 150 µL for 1 hour.
3. Wash plate; Add 25 µL of sample.
4. (Optional wash); Add 25 µL of detection antibody.
5. Wash assay plate; add Read Buffer T; read plate on SECTOR instrument.

Drug Tolerance

Drug interference in immunogenicity assays from free drug in patient samples can cause false negatives and suppressed signal. Assays developed on MSD's robust technology platform demonstrate improved drug tolerance over ELISA methods for many reasons:

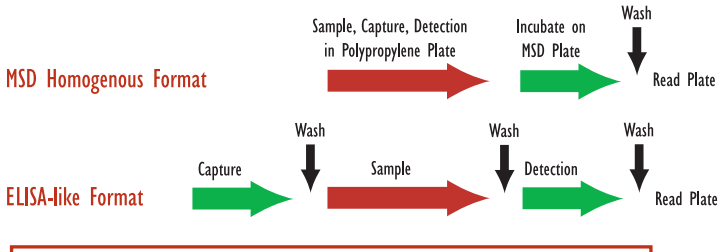
- Homogenous solution phase incubation that is extended overnight
- Improved sensitivity
- Increased surface capacity over ELISA plates
- Increased amounts of biotin and SULFO-TAG labeled antibodies
- Tolerance of ACID/BASE pre-treatment



Drug Tolerance (continued)

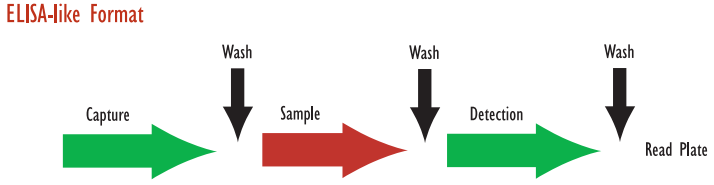
Comparison Without Free Drug Present

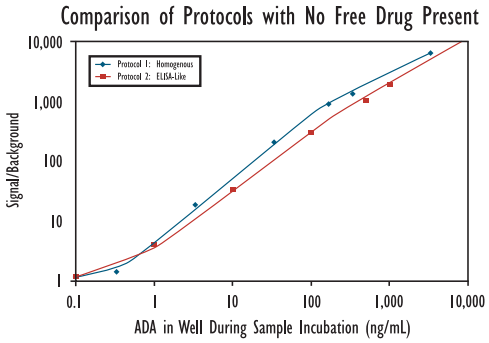
The versatility of MSD plates allows the user to test various assay formats during development and compare performance. MSD bridging assays have improved free drug tolerance over traditional ELISA assays, as previously explained, due to reasons including increased sensitivity and dynamic range. In addition, the choice of protocol format used on the MSD platform also influences drug tolerance, as seen in the comparison below two protocols carried out using MSD plates: MSD's homogenous protocol and an ELISA-like protocol.

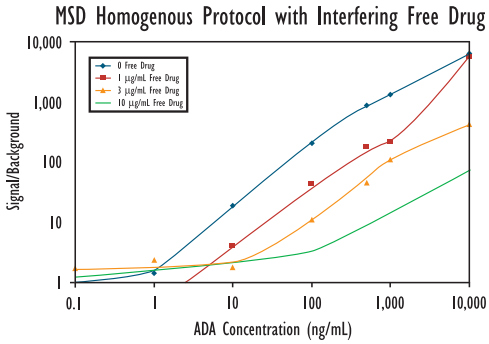


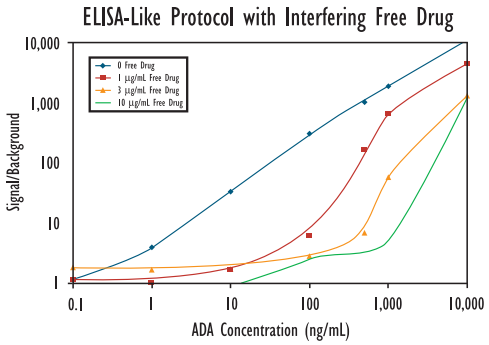
- Independent of assay protocol, the MSD electrochemiluminescent format has a large dynamic range and allows very good sensitivity
- The two formats produce similar results without the presence of free drug

- The MSD homogenous protocol format displays better free drug tolerance at lower anti-drug antibody concentrations
- At high anti-drug antibody concentrations, both protocol formats are comparable



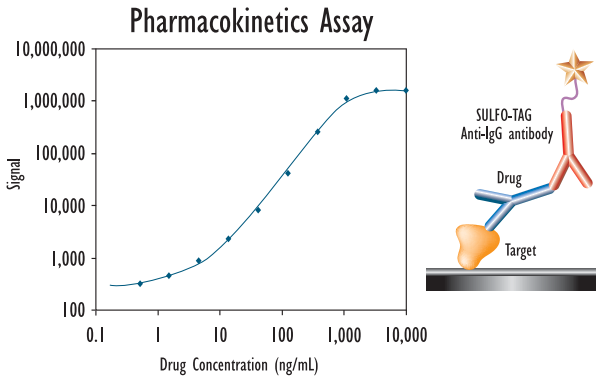






Pharmacokinetics

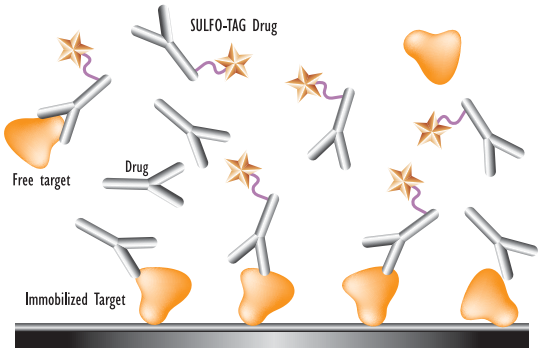
Pharmacokinetics is the study of the metabolism and action of drugs in the body, with emphasis on time course studies of absorption, distribution, period of action, and excretion. Pharmacokinetics assays can easily be implemented on the robust MSD platform using our flexible assay development reagents. MSD assays provide the advantages of less required sample dilutions due to the large dynamic range and greater sensitivity, as well as low matrix interference.

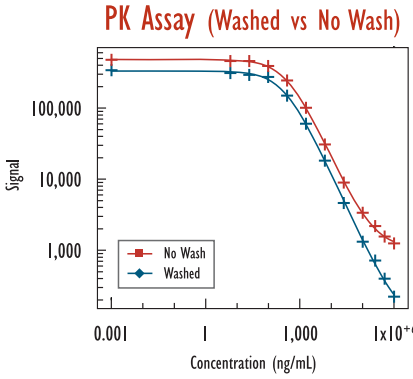


Protocol

1. Coat plate with target. Incubate plate for 1 hour.
2. Add 150 µL/well of Blocker A solution mixed 1:1 with a casein blocker solution. Incubate for 1 hour.
3. Wash plate 3 times. Add 25 µL/well of samples (diluted in mouse serum in example shown). Incubate for 1 hour.
4. Wash plate 3 times. Add 25 µL/well of detection antibody reagent (1 mg/mL SULFO-TAG labeled anti-IgG-specific antibody or unlabeled anti-IgG-specific antibody with 1 µg/mL MSD SULFO-TAG anti-species antibody). Incubate 1 hour.
5. Wash plate 3 times. Add 150 µL/well 1X Read Buffer T, and analyze plate on SECTOR instrument.

Competitive Pharmacokinetic Assay Gives Larger Dynamic Range

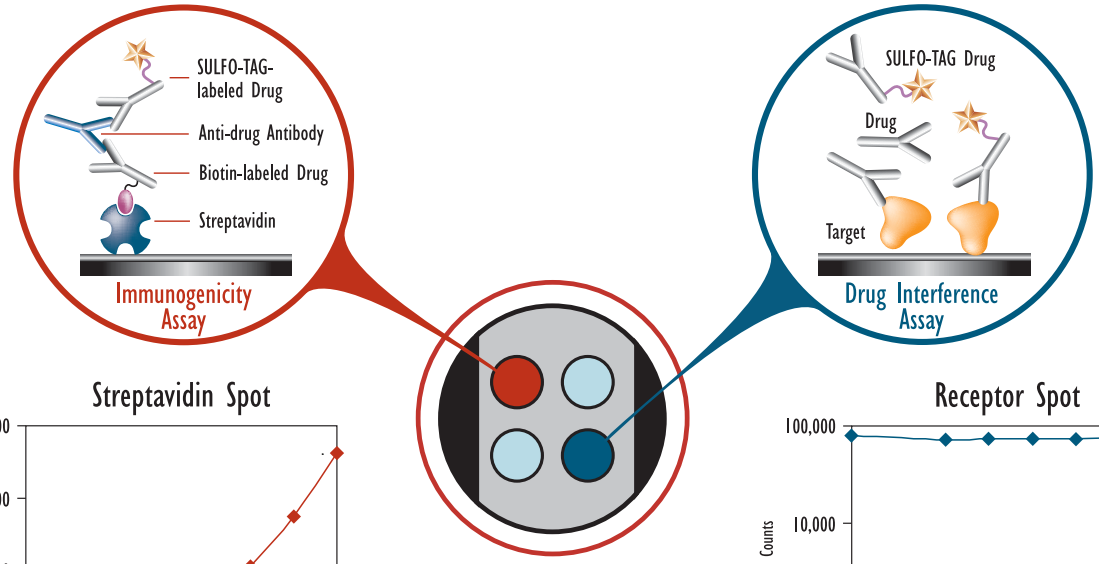




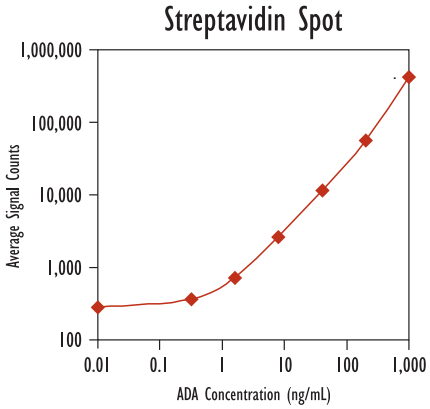
Protocol

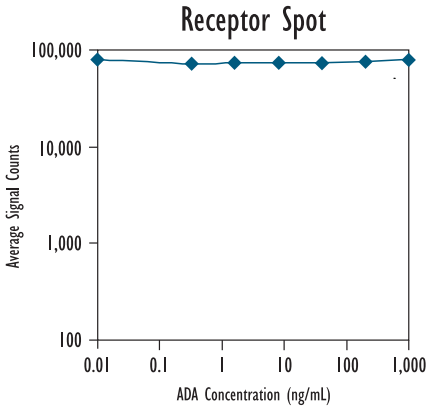
1. Coat plate with target. Wash.
2. Add sample. Incubate for 0-30 minutes.
3. Add 10 µg/mL of SULFO-TAG drug.
4. Add Read Buffer and read.

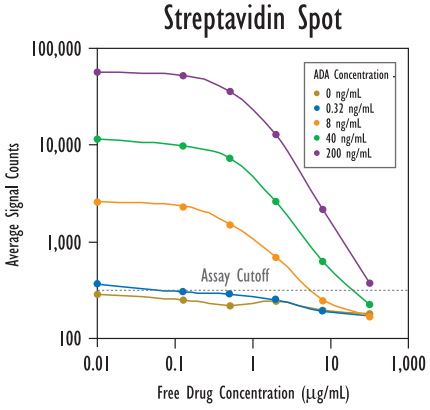
Pharmacokinetics (continued)

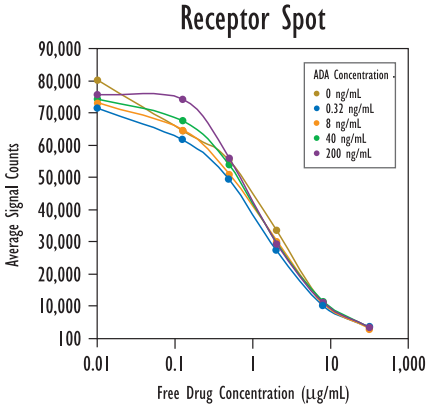


The two assays in the same well appear to act independently in the linear range of the immunogenicity assay.









Free drug suppresses bridging assay, but still detectable at 200 ng/ml with 32 µg/mL of free drug.

The interference assay shows reproducible measurement of free drug interference.

Cell Based Neutralization Assays

Common Problem with CSP-Antibody Binding Assays:

Cell surface proteins can be difficult to purify, or need to be within the cell membrane to look for binding or inhibitory function. Example: 7TM proteins or proteins requiring chaperones.

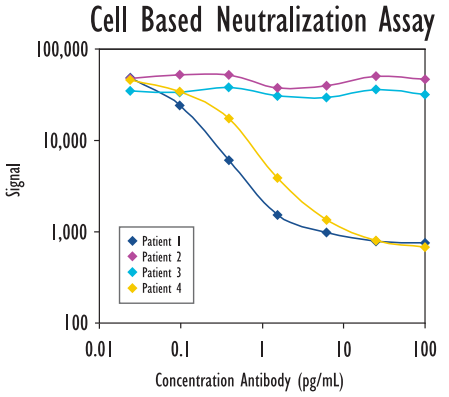
Requirement:

Need to screen with intact receptors in membrane to maintain receptor function and integrity.

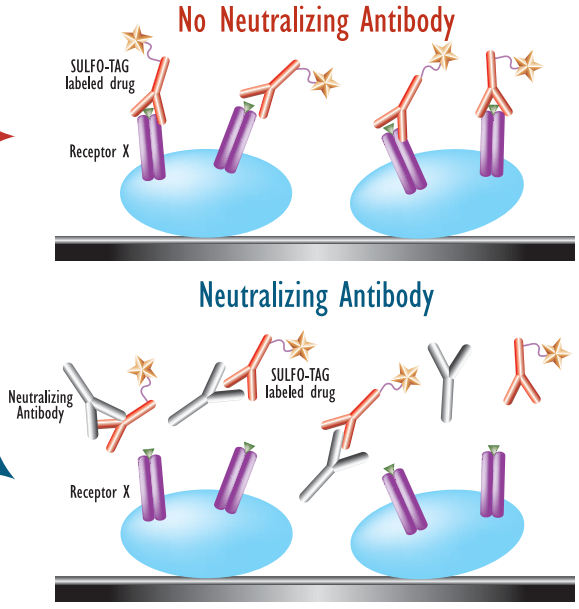
Common Methodology:

Flow Cytometry is used with very low throughput.
Membrane preparations can be difficult to make.
Difficult to solubilize receptors while maintaining active form.

Example: Cell Based Neutralization Assay

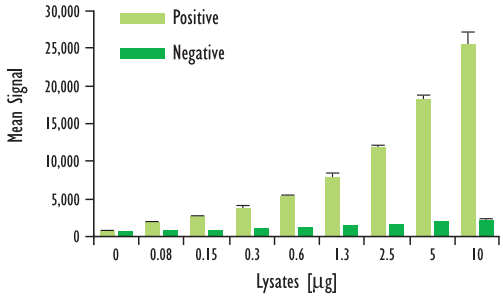


Example data set with 12,500 cells/well. Four different control antibodies were used with 2 antibodies showing neutralizing effects and the other two showing no neutralization.

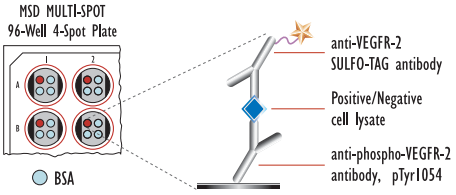


Cell Based Neutralization Assays (continued)

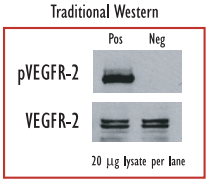
Detection of Phosphorylated VEGFR-2 (Tyr1054/1059) Singleplex Assay

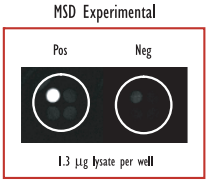


Lysates (µg)	pVEGFR-2 Positive			pVEGFR-2 Negative			P/N
	Average	StdDev	%CV	Average	StdDev	%CV	
0	637	64	10	591	61	10	
0.08	1,803	150	8	719	24	3	2.5
0.15	2,636	170	6	806	39	5	3.3
0.3	3,664	370	10	976	22	2	3.8
0.6	5,317	251	5	1,188	65	5	4.5
1.3	7,806	632	8	1,398	14	1	5.6
2.5	11,831	286	2	1,583	62	4	7.5
5	18,197	608	3	1,918	61	3	9.5
10	25,573	1,704	7	2,130	130	6	12.0



Logarithmically growing HEK293 cells expressing VEGFR-2 (negative) were treated with VEGF (5 min; 1 nM)(positive). Whole cell lysates were added to MSD MULTI-SPOT 4-Spot plates coated with anti-VEGFR-2 antibody on one of the four spatially distinct electrodes per well. Phosphorylated VEGFR-2 was detected with anti-VEGFR-2 antibody labeled with MSD SULFO-TAG reagent.





Conclusions

- MSD Platform provides all the technical advantages of first generation ECL without fluidics, making for a simpler, more robust detection platform.
- The MSD platform has been used at multiple stages of immunogenicity testing including: initial screening, confirmatory assays, neutralization assays, functional assays, isotyping, PK assays, and PD Biomarker Assays.
- MSD immunogenicity assays have been run by several CROs and clinical groups in support of clinical trials.
- Multiplexing capabilities of the MSD platform provide the ability to run in-well controls.
- MSD provides a very flexible system where assays can be performed in multiple different formats, not just bridging assays and required biotin-avidin interactions.
- Over 200 predefined assay kits available for running biomarkers and cell based functional assays.