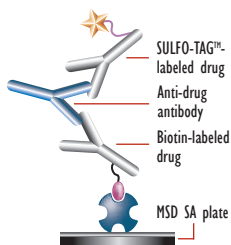
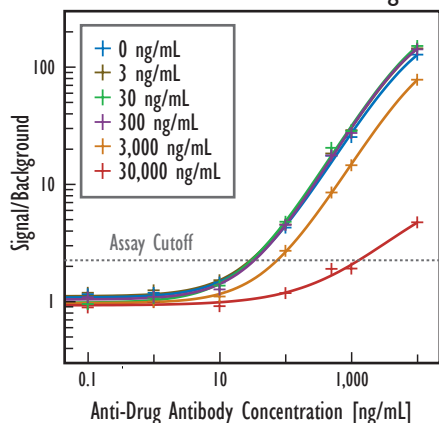


Immunogenicity Assays from Meso Scale Discovery

Immunogenicity testing is a crucial part of biopharmaceutical development. More stringent regulations regarding immunogenicity assay performance necessitates the development of assays exhibiting excellent sensitivity, precision, free drug tolerance and minimal matrix effects. MSD can enable you with assays that meet and exceed all of the criteria listed above, with reagents designed to provide a variety of flexible assay formats. Immunogenicity assays are easily implemented on our low-cost SECTOR™ PR instruments. MSD® SECTOR Imagers, SECTOR PR Readers and their DISCOVERY WORKBENCH® software are GLP-compliant (21 CFR Part 11) with a new data analysis function coming in September 2005. Several contract research labs provide immunogenicity testing on the MSD platform. Contact your area sales representative for more information. Visit www.mesoscale.com for a complete list of MSD products.

Bridging Immunogenicity Assays for rhuMABs

MSD® Bridging Immunogenicity Assay in the Presence of Free Drug



	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

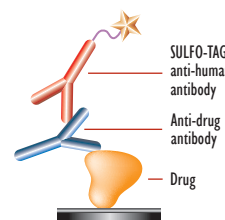
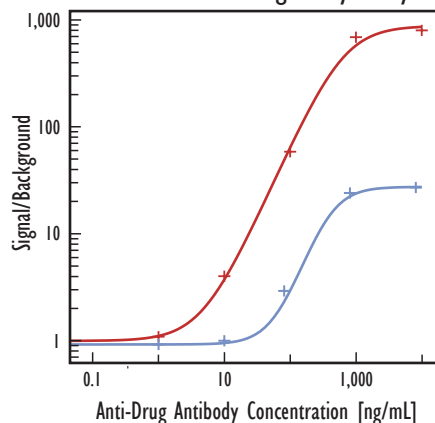
MSD Bridging Assay Protocol

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.

An example of an immunogenicity assay is shown with different levels of free drug added to the sample (see legend). Neat human serum was used as the sample matrix. The LOQ for the assay without free drug was determined to be about 30 ng/mL. There was no effect on the assay for free drug concentrations up to 300 ng/mL. The assay could tolerate up to 1 µg/mL of free drug at 30 ng/mL of ADA.

Sandwich Immunogenicity Assays for Protein Drugs

Comparison of MSD® & ELISA Sandwich Immunogenicity Assays



	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

MSD Sandwich Immunogenicity 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.

Data shown is a comparison of the MSD format (red +) to an ELISA format (blue +). Neat human serum was used as the sample matrix. The top of the curve was about 1 µg/mL for both formats, but the MSD format was 40 times more sensitive.

MSD MULTI-ARRAY® Plates

Bare:

- 96-well, 96-well Small Spot & 384-well* Standard and High-Bind

Avidin:

- 96-well, 384-well* Standard and High-Bind

Streptavidin:

- 96-well and 384-well* Standard and High-Bind

Labeling Reagents & Labeled Reporters

- SULFO-TAG™ NHS-Ester
- SULFO-TAG Streptavidin
- SULFO-TAG Goat-Anti-Human Antibody

For research use only; not for use in diagnostic or therapeutic procedures.

* 384-well plates for SECTOR Imager only

