

Ultra-High Throughput Screening Using Multi-Array 1536-Well Plates

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1 Abstract

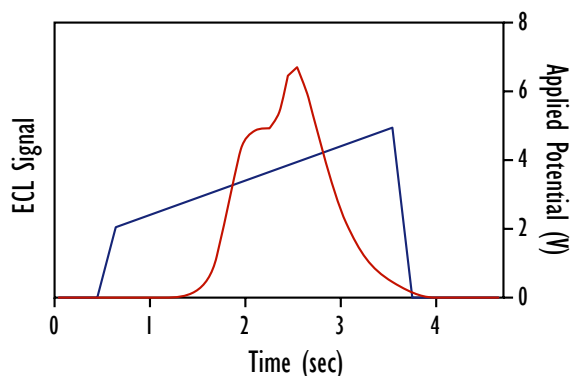
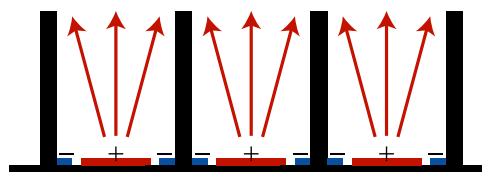
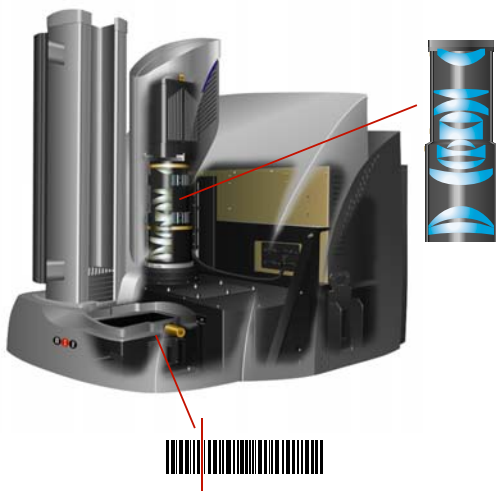
Meso Scale Discovery (MSD) has expanded its technology platform for detection of biomolecules and other analytes to include Multi-Array™ 1536-well plates. These plates allow for experimentation and screening in ultra high throughput modes with a per plate read time of approximately 2 minutes. Similar to other MSD Multi-Array plates, the 1536-well plates are available with different surface properties, have high sensitivity (<20 attomoles), have a wide dynamic range (>5 logs) with less than 0.2% well-to-well optical cross-talk, and are compatible with most lab automation equipment. Assays developed on Multi-Array 384-well plates were transferred to 1536-well plates for a number of different assays including protein-protein binding, competitive and sandwich immunoassays, and receptor-ligand binding. We present screening data with workflows capable of greater than 200,000 data points per shift.



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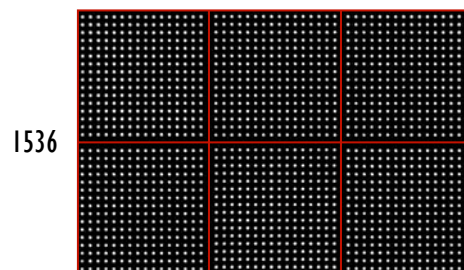
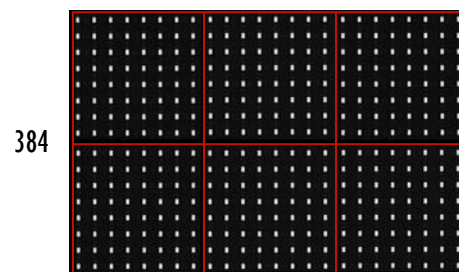
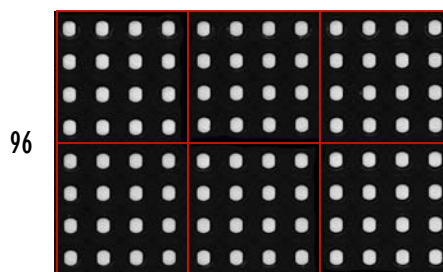
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2 SECTOR™ Imager 6000 and Electrochemiluminescence



ECL signal generated from surface-bound MSD TAG™ by applying a potential ramp (2–5 V).

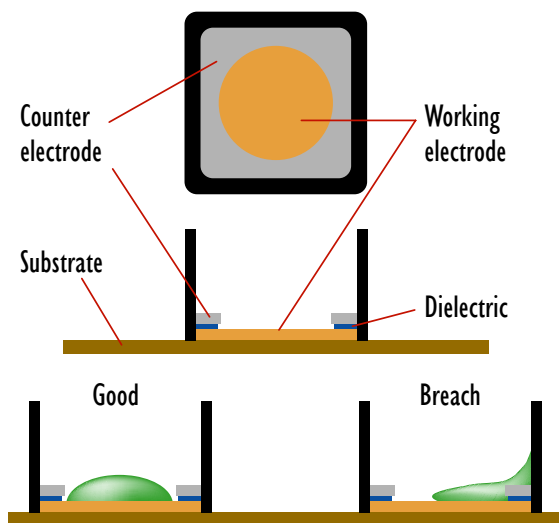
- Highly sensitive electrochemiluminescent detection
- Custom optics with telecentric lens design and CCD imaging detection
- Windows 2000-based, GLP-compliant software (21 CFR Part 11)
- Designed for high-throughput screening (HTS) and proteomic studies
- Compatible with 96-, 384-, 1536-well and Multi-Spot (24- and 96-well) custom plates
- ~2 minutes per plate, format independent
- Fully robotically compatible



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3 Plate Geometries Minimize Reagent Use and Maximize Assay Efficiency



Centered dispenses with appropriate volumes and buffers will optimally cover the working electrode surfaces on 1536-well plates.

Signal is generated at the working electrode; therefore, the most efficient and sensitive approach involves confining capture reagents to the working electrode. For example, capture antibody is spotted on the working electrode to localize specific analyte binding in a detectable region and to minimize binding of the analyte to other surfaces. The capture molecule can passively adsorb to the uncoated working electrode.

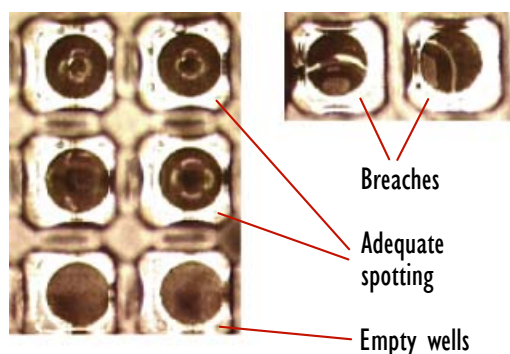
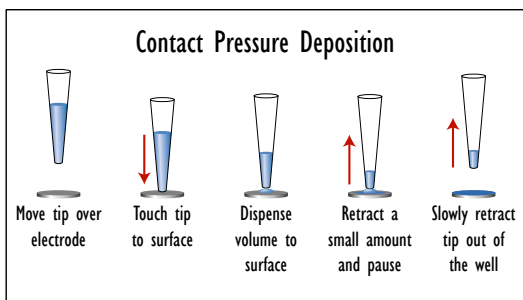
When the capture molecule is immobilized outside the working electrode, analyte may be bound unproductively to surfaces other than the working electrode leading to less analyte detected at the working electrode. Ideal spotting completely fills the working electrode without going beyond the edge of the working electrode. Some solutions form a readily visible “dome” when spotted successfully. The counter electrode in the 1536-well plate and the dielectric in the 384-well plate helps to contain the fluid on the working electrode.



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4 1536-well Pipetting Techniques With Standard Automation



Multi-Array 1536-well plates can be used with standard lab automation equipment.

Liquid handling has been a problem in dealing with 1536-well plates. It often requires custom automation. We use standard automation equipment with our plates and have developed techniques on both the Biomek FX™ and the CyBio-Well vario™ for spotting fluid to the electrode surface. We have been able to dispense as low as 0.25 µl of fluid into dry plates. The CVs for these pipetting steps are between 10% and 20%, but the assay CVs can be much lower.

Spotting and pipetting methods have been developed for Multi-Array 384-well and 1536-well plates that exploit the features of these plates and create highly sensitive assays. Different methods are optimal for different assays, and the effectiveness of the method is determined by a number of factors including plate surface type, reagent/buffer properties, and solution volumes. The “Contact Pressure” spotting method that has been used successfully in a number of applications is depicted here as an example. Photographs of properly and poorly spotted wells are also shown.



Beckman Coulter
Biomek FX™



CyBio AG
CyBio-Well vario™

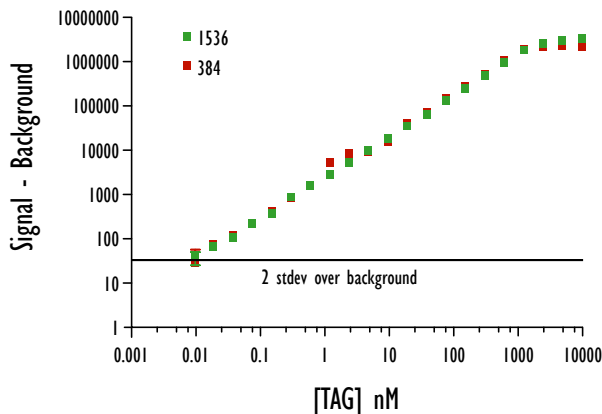


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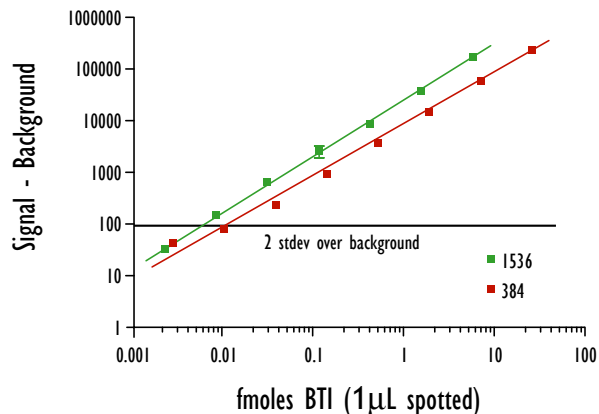
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5 1536 Multi-Array Plates: Dynamic range and sensitivity for detecting TAG

Detection of free MSD TAG has a linear dynamic range of > 5 logs



Detection of MSD TAG-labeled biotin IgG spotted on the working electrode



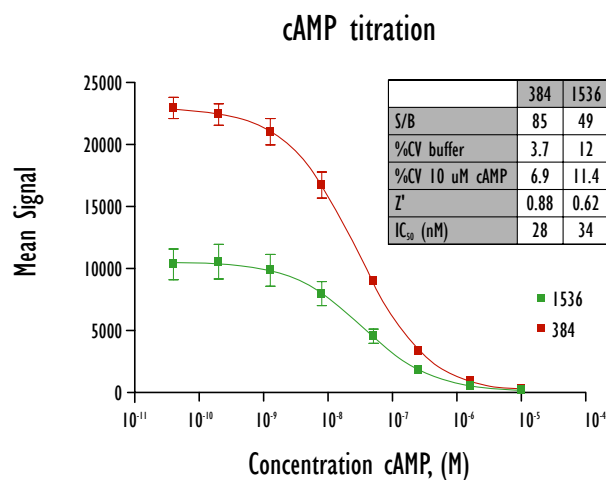
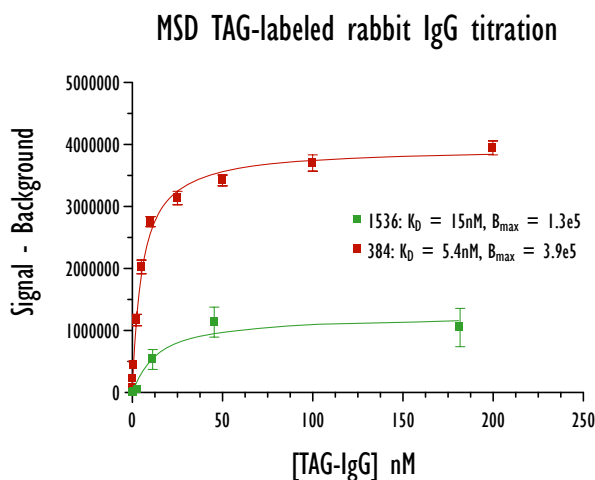
Multi-Array 1536-well plates were evaluated and compared to 384-well plates using MSD TAG in solution and immobilized on the electrode surface as MSD TAG-labeled biotin-IgG (BTI). Using the assumption that all of the BTI (~3 TAG labels per protein) is immobilized, an upper limit for the detection limit for TAG molecules on 1536-well plates is ~10-20 attomoles of TAG (6-12 million molecules). Error bars are shown or are less than the height of the data symbol. Optical crosstalk was measured to be less than 0.2% using a single point near saturation.



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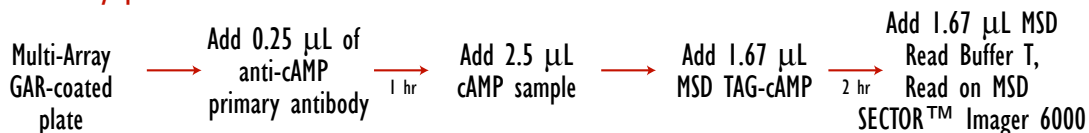
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6 Demonstration of a protein-protein binding assay and competitive immunoassay transferred from 384-well to 1536-well format



Multi-Array 384-well and 1536-well plates coated with goat anti-rabbit IgG (GAR) were evaluated by detecting specific binding of MSD TAG-labeled rabbit IgG (left panel). Background signal was obtained from wells with no GAR. The two plate types showed comparable binding affinities. GAR coated plates were also used to detect cyclic AMP (cAMP) in a competitive immunoassay using MSD TAG-labeled cAMP (see poster "A Multi-Array Technology Based Assay for cAMP"). The 1536-well format was developed by directly scaling the volume of the 384-well format. IC_{50} values are comparable for 384- and 1536-well plates with high S/B and good Z' scores. Each data point of the 1536-well data was determined from 32 replicates. This format can provide significant reduction in sample and reagent consumption. The format allows for the processing of 30 1536-well plates per batch and as many as 5 batches per shift.

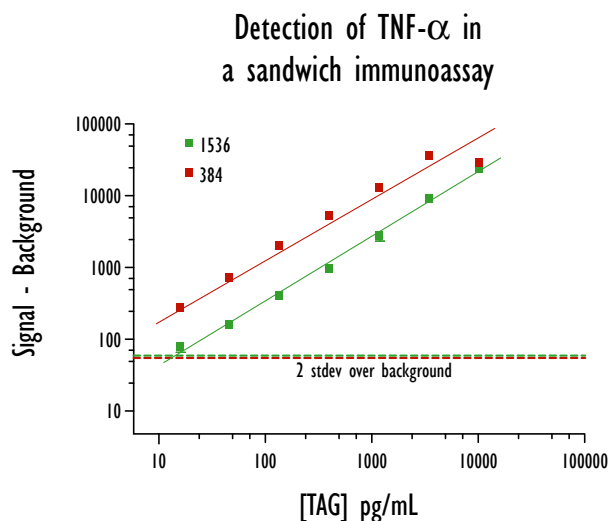
cAMP assay protocol for 1536-well:



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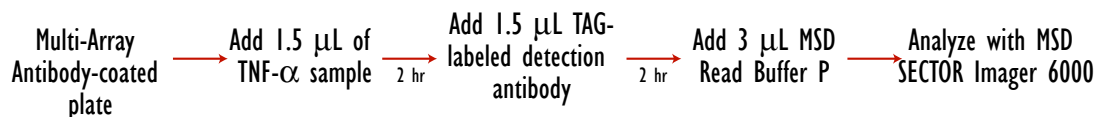
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7 Demonstration of sensitive cytokine detection in a sandwich immunoassay transferred from 384-well to 1536-well format



Antibody was immobilized on 384- and 1536-well plates and used to detect binding of the cytokine, tumor necrosis factor α (TNF- α). Background signal was obtained from wells with no capture antibody. TNF- α was detected by the binding of MSD TAG-labeled anti-TNF- α detection antibody. The 1536-well format showed comparable sensitivity to the 384-well format, detecting from 14-10,000 pg/mL with a linear dose response. At the 14 pg/mL level, no more than 1.2 attomoles ($\sim 750,000$ molecules) of TNF- α were present in the well on the 1536-well plate.

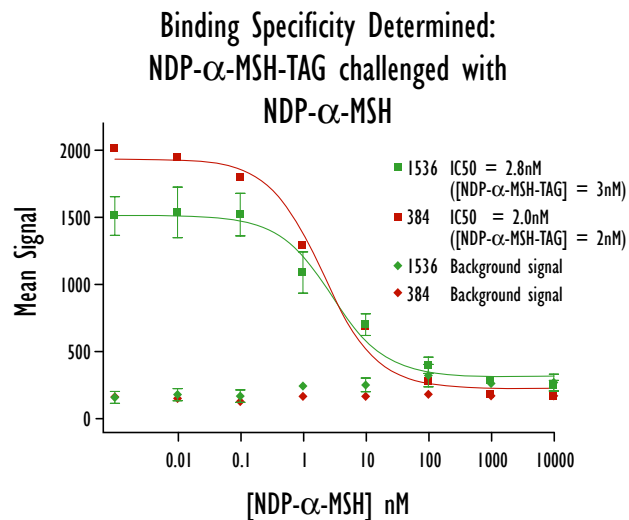
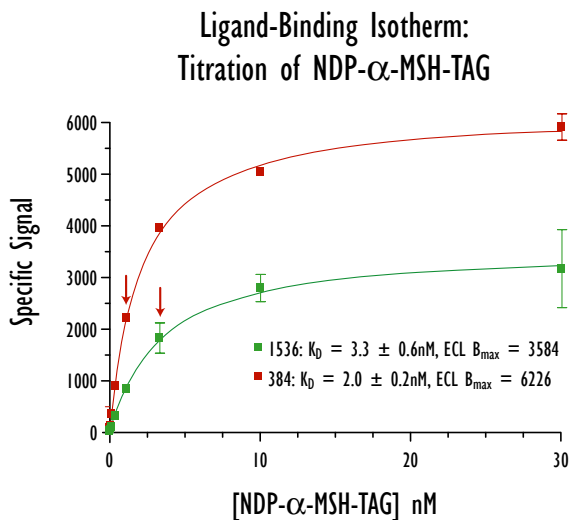
Cytokine detection assay protocol for 1536-well:



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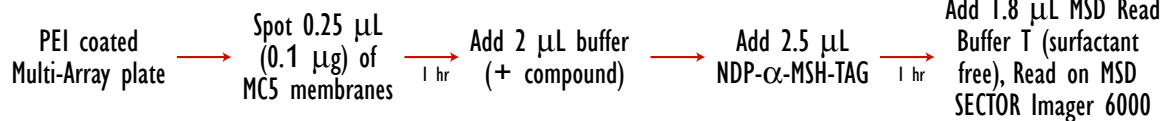
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8 Demonstration of G-protein coupled receptor — ligand binding assay transferred from 384-well to 1536-well format



HEK293 cell membranes containing the melanocortin subtype 5 (MC5) receptor were immobilized on Multi-Array 384-well and 1536-well plates and used to detect specific binding of the MSD TAG-labeled ligand, NDP- α -MSH. Background signal was obtained from the parent cell line. K_D values of ~ 2 -3nM agree with published values, and binding specificity of the receptor for the labeled ligand was demonstrated with competition studies using unlabeled NDP- α -MSH. S/B ratios at the K_D of this interaction were 7.5 (1536-well) and 13 (384-well) at the NDP- α -MSH-TAG concentrations used for screening (red arrows).

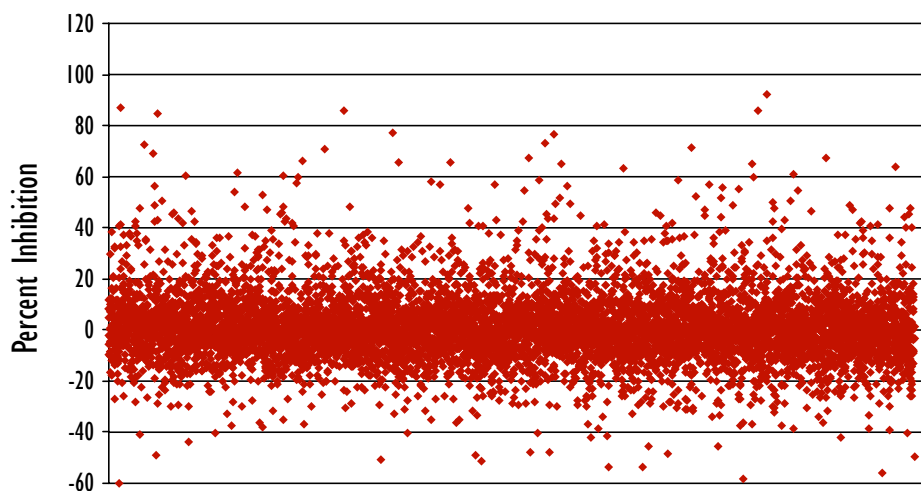
MC5 assay protocol for MSD 1536-well Multi-Array Plates:



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9 Screen of 8000 compounds in 1536-well format



A screen of 8000 compounds was carried out on the MC5 assay in MSD Multi-Array 1536-well plates. The average S/B ratio was 7.8, and the average of the Z' scores for each plate across the screen was 0.53. The hit rate for inhibitors of $>50\%$ was 0.5%. This was similar to a comparable screen done in 384-well format where the Z' score averaged 0.74 and the hit rate was 0.38%.

The 1536-well format affords considerable time and reagent savings. At a read time of ~ 2 minutes per plate, it is feasible to process 30 plates or 46,080 data points per hour. The SECTOR Imager 6000 has been shown to be integrated with multiple robots and track systems. In a fully integrated automation system, greater than 300 plates can be processed in a single day. Because of the lower well volumes, valuable reagents such as compounds and biomolecules can easily be reduced five-fold.



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10 Conclusions

The 1536-well platform enables assays with reduced consumption of reagents and significant time savings with a dynamic range of >5 logs and very high sensitivity.

Optimized pipetting and liquid handling techniques that leverage off of the MSD platform have been developed for the 1536-well plates using standard lab automation equipment. Usability with standard pipetting automation such as the Biomek FX and CyBi-Well vario allows for easier assay development in the 1536-well format, and usability in non-UHTE labs.

Multiple assays developed and optimized on 384-well plates were transferred and validated on 1536-well Multi-Array plates. Little optimization was required when transferring from 384-well to 1536-well. There is typically a 2-4 fold increase in throughput when switching from the 384-well format to the 1536-well format.

While the absolute signal levels are different between the 1536-well and 384-well plates, the assay performances are comparable. Both cAMP and MC5 assays show the similar IC_{50} values for the two plate formats with good precision. The binding affinity K_d for the MC5 receptor ligand determined on the 1536-well format is within 2 fold of that determined on the 384-well format and published values.

A GPCR assay (MC5) was screened with 8000 compounds. The average S/B was 7.8, the average Z' score was 0.53, and the hit rate for $>50\%$ inhibitors was 0.5%.



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