MSD MULTI-SPOT® 384-Well 4 Spot Format: Higher Content and Throughput with Less Sample

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MULTI-SPOT® 384-Well 4 Spot products from Meso Scale Discovery combine the high throughput advantage of singleplex MULTI-ARRAY™ 384-Well plates with the high content of MULTI-SPOT 96-Well 4 Spot plates, achieving similar information at high rates of measurement with less sample. Assays are readily transferred from other formats to the 384-Well 4 Spot format enabling easy transition to high throughput paradigms. The performance of assays in the 384-Well 4 Spot format is comparable to the 96-Well 4 spot format, with similar signals and sensitivities. Likewise, workflows for 384-Well 4 Spot assays are analogous to other MSD assay formats with limited (or no) wash steps recommended and short (1-2 hour) incubations. A typical incubation volume suggested for the MSD 384-Well 4 Spot format is 10 µL, which permits conservation of valuable samples and reagents relative to most 96-Well formats and other technology platforms. MSD is now offering custom 384-Well 4 Spot kits in 5- and 50-plate configurations that include all materials and reagents needed to rapidly execute high throughput measurements. Here we present several assay panels on the 384-Well 4 Spot format in order to demonstrate its utility across a wide range of biological applications including quantitative detection of cytokines, serum biomarkers, and cell signaling markers. Each example illustrates the high performance and wide dynamic range achievable in this unique format.

MSD MULTI-ARRAY Technology and MULTI-SPOT Plates

Instrument Features

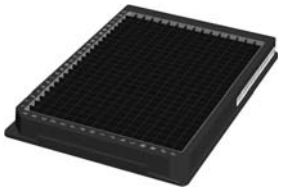
- Cooled CCD camera for low background and large dynamic range
- Fast read time, ~1 minute for all plate types
- Easy to use, no filters or end-user adjustments
- On board data analysis tools
- Integrated stackers for walk-away operation

SECTOR™ PR 400

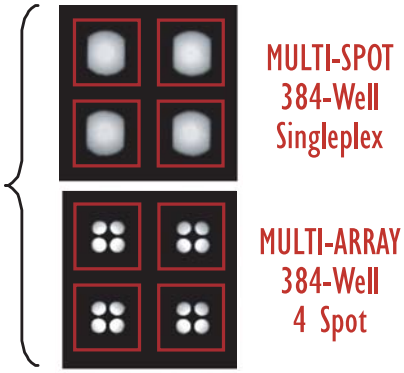
SECTOR Imager 6000

Plate Features

- Disposable plates
- Patterned arrays for maximum flexibility
- Multiplexing in 96- and 384-well formats
- Compatible with automation for high throughput



MSD 384-Well Plate



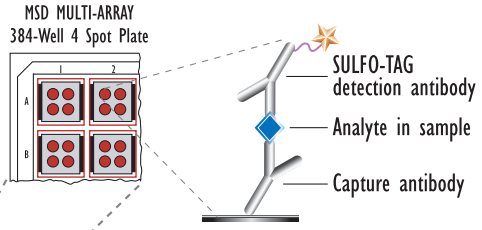
MULTI-SPOT
384-Well
Singleplex

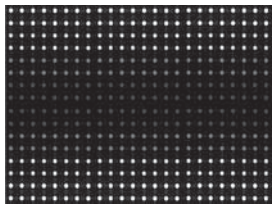
MULTI-ARRAY
384-Well
4 Spot

MULTI-SPOT 384-Well 4 Spot Plates: Higher Content & Throughput

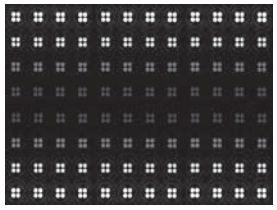
Advantages of MULTI-SPOT 384-Well 4 Spot Plates:

- High throughput: 384 samples per plate
- High content: 4 data points per sample - 1536 data points per plate
- High performance: sensitivity, dynamic range, and specificity
- Reduced sample requirement: 10 µL/well for 384-Well plates
- Easy workflow: similiar incubation times to 96-Well format
- Suitable for use in ordinary lab settings: no special volume, liquid handling, or equipment requirements

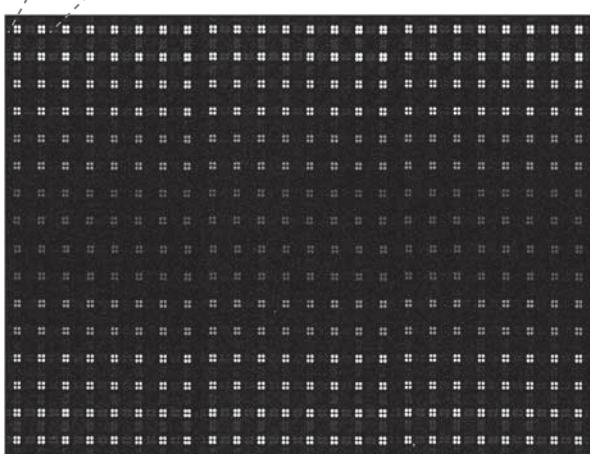




MSD 384-Well Plates (High Throughput)

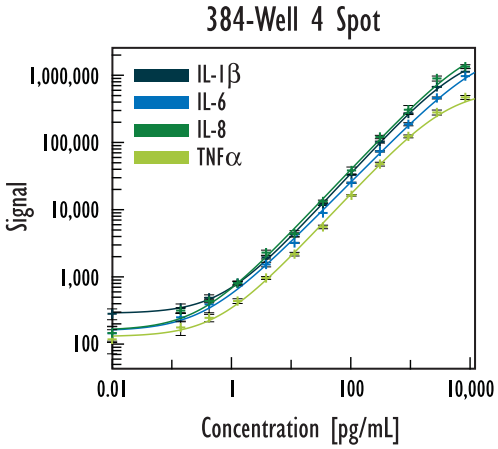


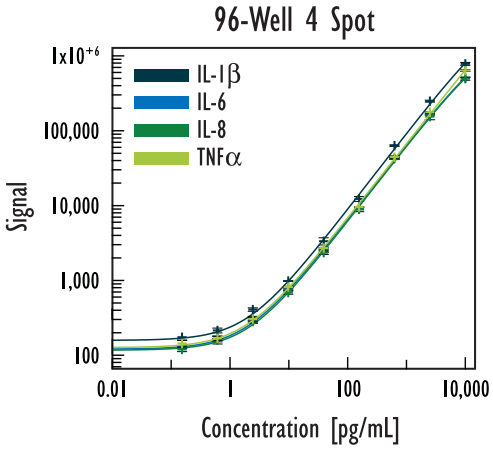
MSD 96-Well 4 Spot Plates (High Content)

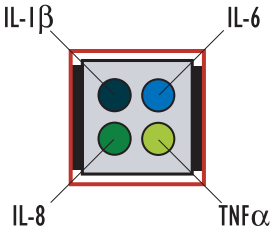


MSD 384-Well 4 Spot Plates (High Throughput & High Content)

Cytokine Application: Human Pro-Inflammatory 4-Plex Panel II





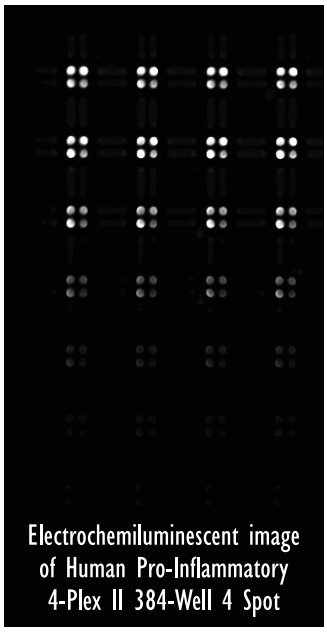


	Detection Limit (pg/mL)	
	384-Well 4 Spot	96-Well 4 Spot
IL-1β	0.3	0.5
IL-6	0.2	0.6
IL-8	0.2	0.5
TNFα	0.5	0.4

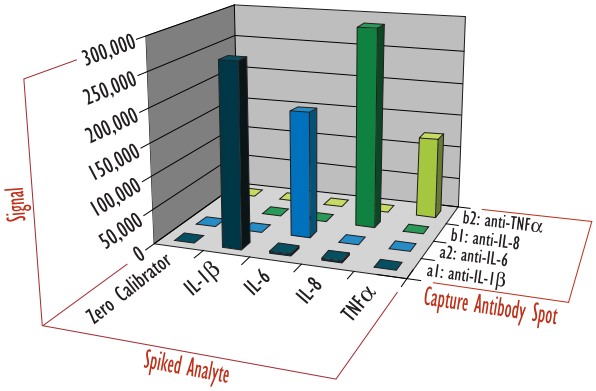
Detection limit is 2.5 StdDev above background

The performance of multiplexed human cytokine assays on MULTI-SPOT 384-Well 4 Spot plates is comparable to those on MULTI-ARRAY and MULTI-SPOT 96-Well 4 Spot plates. Titration curves show dynamic range windows of 3-4 logs and detection limits of less than 1 pg/mL for IL-1β, IL-6, IL-8, and TNF-α. The above titration curves are using a tissue culture protocol with each point measured in quadruplicate; variability is low, and the average CV above the detection limit is ≤ 8%. Measurement of the signal on all spots when individual calibrators are used as samples allows for an assessment of assay specificity. Using a spike of 925 pg/mL calibrator, the cross reactivity between spots is less than 0.5% for all combinations; this is similiar to that observed on the 96-Well 4 Spot format. Recommended protocols for 384-Well 4 Spot cytokine assays are the same as those suggested for 96-Well 4 Spot assays except less sample (10 µL) is required.

Specificity of Human IL-1β, IL-6, IL-8, & TNFα Assays on 384-Well 4 Spot



Electrochemiluminescent image of Human Pro-Inflammatory 4-Plex II 384-Well 4 Spot



Cross reactivity is defined as: (signal in the presence of the interferent – signal in the absence of interferent) / interferent signal.

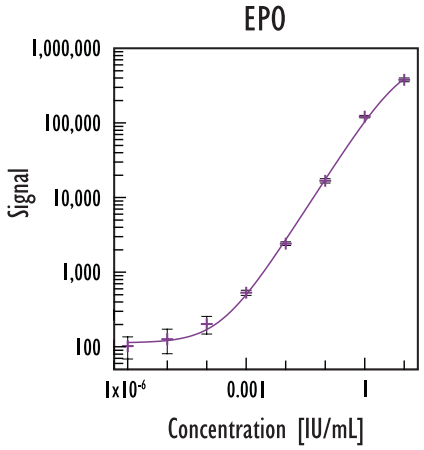
% Cross Reactivity

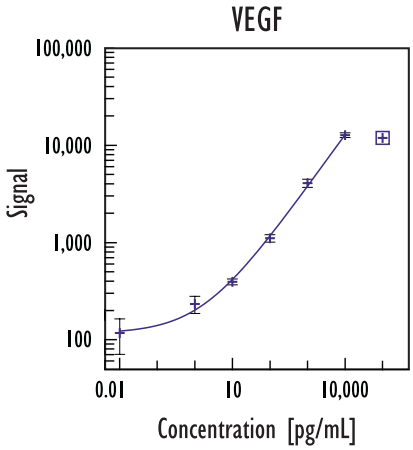
	Calibrator			
	IL-1β	IL-6	IL-8	TNF
a1: anti-IL-1β	100	0.4	0.3	0.2
a2: anti-IL-6	0.1	100	0.07	0.2
b1: anti-IL-8	0.2	0.3	100	0.3
b2: anti-TNFα	0.02	0.1	0.1	100

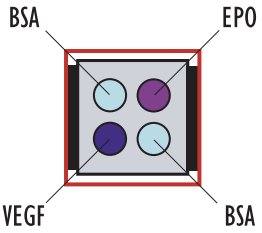
384-Well 4 Spot Cytokine Tissue Culture Protocol

- Add 10 µL of calibrator or sample per well, incubate at RT with shaking, 2 hours
- Add 10 µL diluted MSD SULFO-TAG-labeled detection antibody per well, incubate at RT with shaking, 2 hours
- Wash plates with PBST
- Add 40 µL MSD Read Buffer T
- Analyze with SECTOR Imager

Hypoxia Markers in 384-Well 4 Spot Format: Human EPO/VEGF Duplex Assay





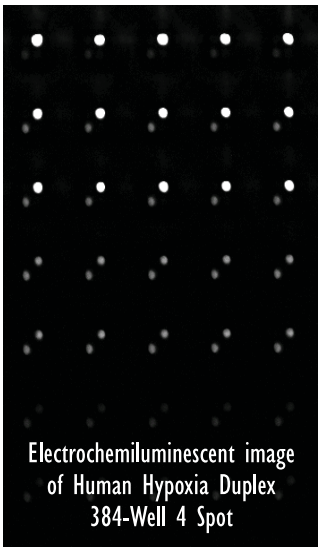


	Detection Limit (pg/mL)	
	384-Well 4 Spot	96-Well 4 Spot
EPO	0.0002 IU/mL	0.0001 IU/mL
VEGF	2 pg/mL	0.8 pg/mL

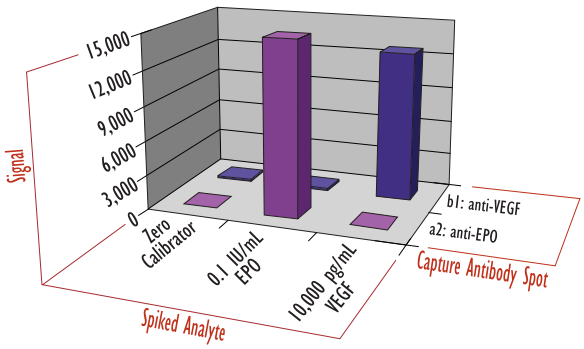
Detection limit is 2.5 StdDev above background

Multiplexed serum biomarker assays also can be performed on MULTI-SPOT 384-Well 4 Spot plates. The above example demonstrates the measurement of the human hypoxia markers erythropoetin (EPO) and vascular endothelial growth factor (VEGF) in tissue culture medium. Performance in the 384-Well 4 Spot format is similar to the 96-Well 4 Spot format with dynamic range, sensitivity, specificity, and variability all being comparable. The above titration curves are determined from 12 replicates per point; variability is low, and the average CV above the detection limit is $\leq$ 7%. Measurement of the signal on both spots when individual calibrators are used as samples allows for an assessment of assay specificity. Using spikes of 10 ng/mL VEGF and 100 mIU/mL of EPO, the cross reactivity between spots is less than 0.5%; similiar to that observed on the 96-Well Human Hypoxia Duplex. Recommended protocols for 384-Well 4 Spot serum biomarker assays are similar to those suggested for 96-Well 4 Spot assays except less sample (10 µL) is required.

Specificity of Human EPO & VEGF Assays on MULTI-SPOT 384-Well Hypoxia Duplex



Electrochemiluminescent image of Human Hypoxia Duplex 384-Well 4 Spot



Cross reactivity is defined as: (signal in the presence of the interferent – signal in the absence of interferent) / interferent signal.

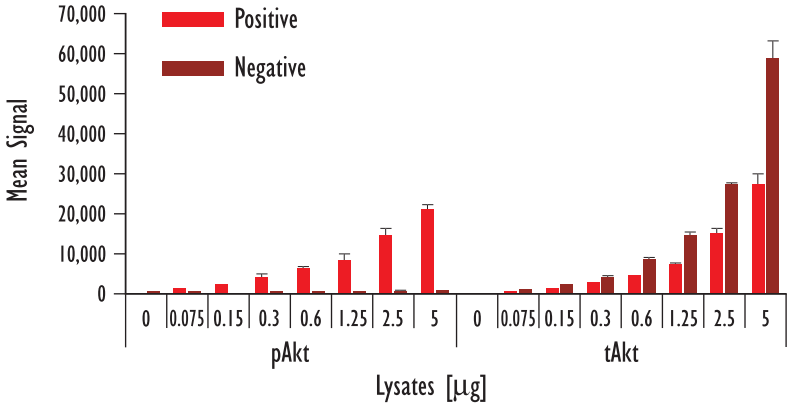
% Cross Reactivity

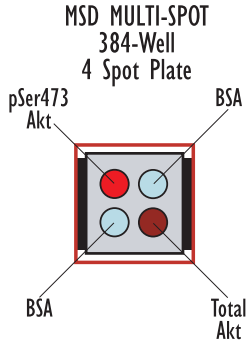
	EPO only	VEGF only
a2: anti-EPO	100	0.10
b1: anti-VEGF	0.04	100

384-Well Human Hypoxia Duplex - Tissue Culture Protocol

- Add 40 µL blocking solution per well, incubate at RT with shaking, 1 hour
- Wash plates with PBST
- Add 10 µL of diluted MSD SULFO-TAG labeled detection antibody per well
- Add 10 µL of calibrator or sample per well, incubate at RT with shaking, 4 hours
- Wash plates with PBST
- Add 40 µL MSD Read Buffer T
- Analyze with SECTOR Imager

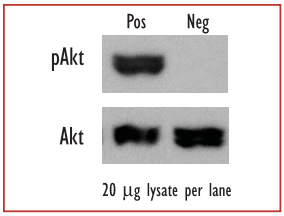
Cell Signaling Application: Phosphorylated & Total Akt in Cell Lysates



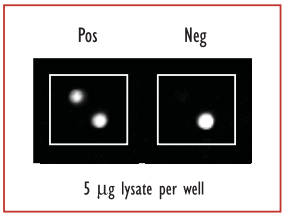


	Lysates (µg)	pAkt Positive			pAkt Negative			P/N
		Average	StdDev	%CV	Average	StdDev	%CV	
pAkt	0	162	27	17	247	50	20	
	0.075	1,150	166	14	232	68	29	5.0
	0.15	2,162	145	7	215	36	17	10.0
	0.3	4,106	928	23	277	85	31	14.8
	0.6	6,193	548	9	376	32	8	16.5
	1.25	8,194	1,662	20	443	41	9	18.5
	2.5	14,716	1,668	11	616	71	12	23.9
	5	20,710	1,654	8	710	93	13	29.2
tAkt	0	84	43	52	127	45	36	
	0.075	616	63	10	1,064	68	6	0.6
	0.15	1,320	126	10	2,217	175	8	0.6
	0.3	2,530	105	4	4,217	119	3	0.6
	0.6	4,444	200	5	8,814	445	5	0.5
	1.25	7,254	612	8	14,531	1,127	8	0.5
	2.5	14,923	1,278	9	27,269	605	2	0.5
	5	27,371	2,606	10	58,860	4,175	7	0.5

Traditional Western



MSD Experimental



Multiplexed phosphoprotein assays can be performed on MULTI-SPOT 384-Well 4 Spot plates to measure modification and abundance of cell signaling markers in high throughput. The above example demonstrates the measurement of phosphorylated and total Akt in cell lysates. Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 µM; 2.25 hr)(negative). Whole cell lysates were added to 384-well MSD MULTI-SPOT 4 Spot plates coated with anti-phospho-Akt antibody and anti-total-Akt antibody on two of the four spatially distinct electrodes per well. Phosphorylated and total Akt were detected with anti-total-Akt antibody labeled with MSD SULFO-TAG reagent.

Phosphoproteins and cell signaling markers can be detected at very low levels using the 384-Well 4 Spot format. In the 384-Well Phosphorylated and Total Akt Duplex, Akt is readily detected from lysates containing 0.1 µg of protein which is typically within the range of protein one would obtain from culturing adherent cells in a 384-Well plate. In the above lysate titrations each point is measured with 4 replicates, and the average CV is ≤ 13%. Recommended protocols for 384-Well 4 Spot phosphoprotein assays are similar to those suggested for 96-Well 4 Spot assays except a smaller sample volume (10 µL) is utilized. Incubation times are similar to those used for 96-Well formats but they may vary with analyte.

384-Well Phosphorylated and Total Akt Duplex Protocol

- Add 40 µL blocking solution per well, incubate at RT with shaking, 1 hour
- Wash plates with TBST
- Add 10 µL of diluted lysates per well, incubate at RT with shaking, 1 hour
- Wash plates with TBST
- Add 10 µL of diluted MSD SULFO-TAG-labeled detection antibody; incubate at RT with shaking, 1 hour
- Wash plates with TBST
- Add 40 µL MSD Read Buffer
- Analyze with SECTOR Imager

384-Well 4 Spot Kits

Kit Components

- Pre-coated MULTI-SPOT plate: contains patterned array of capture antibodies
- Detection Antibody: MSD SULFO-TAG labeled detection antibodies
- MSD Read Buffer: buffer optimized for ECL measurement
- Set of Calibrators: for calibration curve to quantitate analyte (if applicable)
- Optimized protocol
- Diluents and blockers needed for optimal performance

Ordering information for Multiplexed 384-Well Kits

Kit Size	Catalog #	Description
5 plate	K21025B-2	MS6000 Human ProInflammatory-4 II 384 Tissue Culture Kit
50 plates	K21025B-6	MS6000 Human ProInflammatory-4 II 384 Tissue Culture Kit
5 plates	K21027B-2	MS6000 Human Hypoxia 384 Tissue Culture Kit
50 plates	K21027B-6	MS6000 Human Hypoxia 384 Tissue Culture Kit

Available for use with the SECTOR Imager 6000

Conclusions

- MSD's 384-Well 4 Spot platform is unique in its ability to simultaneously provide high throughput and high content
- Performance, specificity, and dynamic range of the 384-Well 4 Spot format are comparable to the 96-Well 4 Spot format
- The utility of 384-Well MULTI-SPOT arrays has been demonstrated for a wide variety of biological applications including cytokines, serum biomarkers, and cell signaling markers
- Reduced assay volume requirement allows for conservation of valuable samples and reagents (10 µL instead of 25 µL for typical 96-well MSD assays)
- 1536 data points obtained per plate
- 384-Well 4 Spot plates are suitable for use in typical biological laboratory settings
    - Assay volumes are manageable without special considerations required for other high throughput formats (such as 1536-well plates) – assays can still be run "by hand"
    - Ordinary laboratory liquid handling equipment is suitable
    - Typical plate shakers effectively move liquid in wells so that assays are not limited to mass transport kinetics
    - Assay workflows (protocols) are similar to 96-well and 384-well plate formats
    - No special considerations for evaporation
    - More reasonable surface to volume ratio than 1536-well plates
- MSD assays can be transferred readily from other plate formats to 384-Well 4 Spot