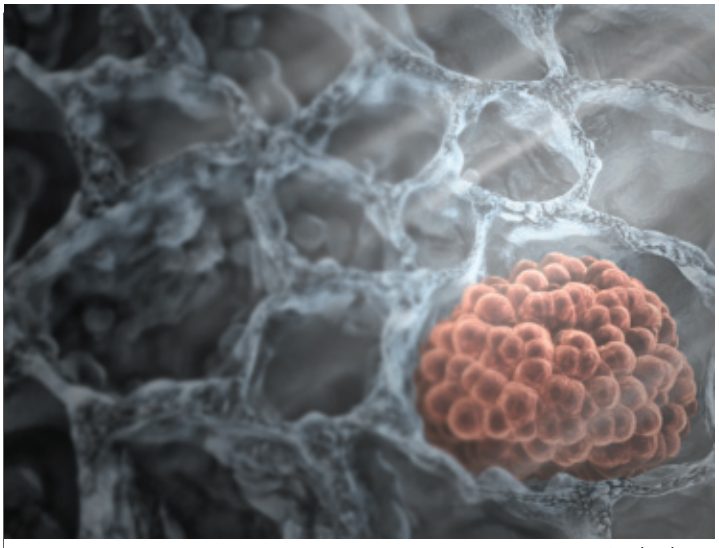
# ONCOLOGY APPLICATIONS

MSD®

# MESO SCALE DISCOVERY® Oncology Applications

MESO SCALE DISCOVERY (MSD®) offers a diverse product line of biomarker assays including many oncology-focused kits. We feature phospho-protein and intracellular signaling assay kits designed for use with whole cell lysates and other complex matrices. Clinical biomarker assays for analysis of serum, plasma, and tissue culture samples are also available from MSD. Whether your focus of study is angiogenesis, apoptosis, or another aspect of oncology research, MSD can enable you with the tools and technology to enhance all stages of cancer therapeutics development.



Cancerous tumor in the lung




MESO SCALE DISCOVERY
A division of Meso Scale Diagnostics, LLC.

9238 Gaither Road
Gaithersburg, MD 20877 USA
Phone: 1.301.947.2085
Fax: 1.301.990.2776
Email: customerservice@mesoscale.com
www.mesoscale.com

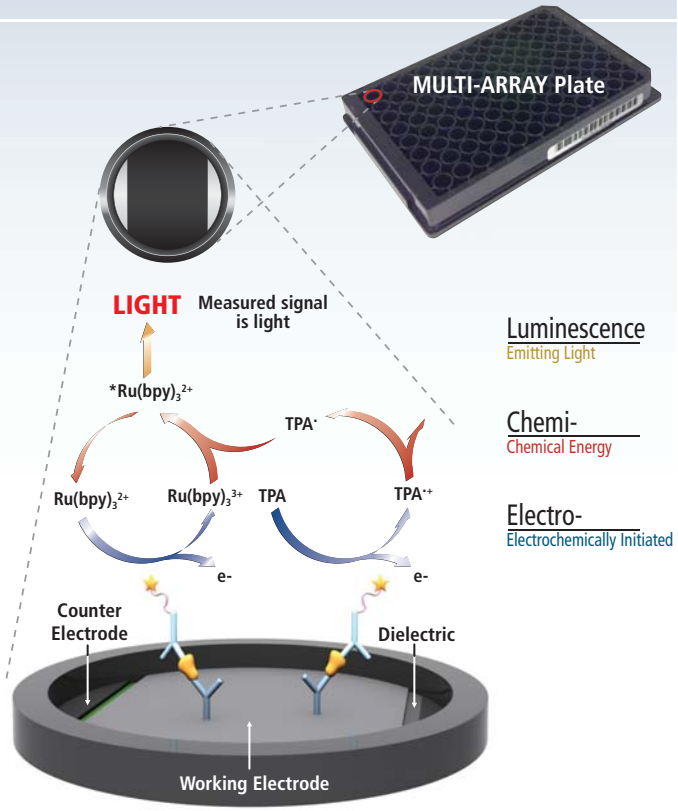

Spot the Difference

# MSD Technology

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels, which 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 conveniently conjugated to biological molecules
- Emission at ~620 nm - eliminates problems with color quenching
- Signal amplification - multiple excitation cycles of each label enhance light levels and improve sensitivity
- Flexible surface coatings to suit most any biology
- Carbon electrode plate surface has 10X greater binding capacity than polystyrene
- Custom surface coatings and patterns




## MULTI-ARRAY and MULTI-SPOT Features:

- Capability to simultaneously measure multiple analytes in the same well
- High density arrays for high throughput multiplexing of biomarkers
- The unique bar code label on each plate enables complete traceability back to MSD manufacturing records
- The MSD DISCOVERY WORKBENCH® software provides customers with a powerful tool for data analysis

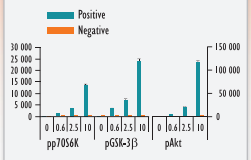

# MSD Multiplex Phosphoprotein Assays:

## Speeding Discovery, Advancing Oncology

## MSD

### Simple and fast

In less than **4.5 hours,** up to 960 quantitative data points can be generated and analyzed.




***Quantitative Result***

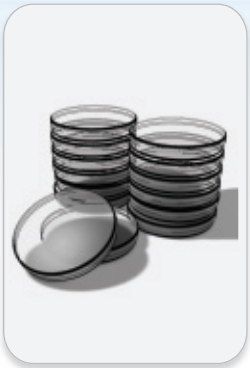
- reduce cost and time
- gain sensitivity
- reduce cell culture volume
- analyze wide range of matrices
- conserve precious samples
- gain with MSD multiplexing
- gain in throughput

**Sample Preparation**
Lysate preparation in 96-well plate and direct transfer to MSD plate

- Multiple phosphoproteins as well as total pools can be accurately quantified in a single well from as few as 1000 cells
- % Phosphorylation of protein can also be calculated

## Western Blot

### Laborious and time consuming

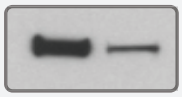


**Sample Preparation**

**Gel Electrophoresis**
6 gels ~ equivalent to one 96-well plate

**Blotting and transfer to membrane**

With Western blot, more than **10 hours, and up to 2 days** may be required to analyze 10-fold less data.



***Non-quantitative Result***

- Need expensive imaging system for semi-quantitative analysis
- Low throughput: stripping and reprobing are an inaccurate and undesirable approach for multiplexing

# The Challenge:

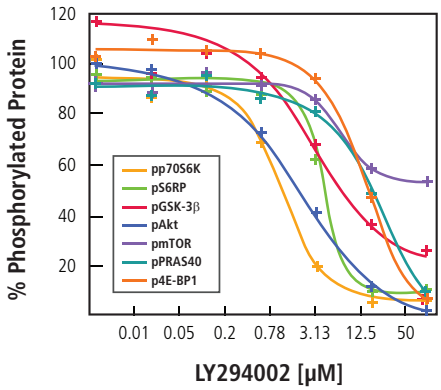
## Rapid Discovery to Advance Promising Compounds in Oncology

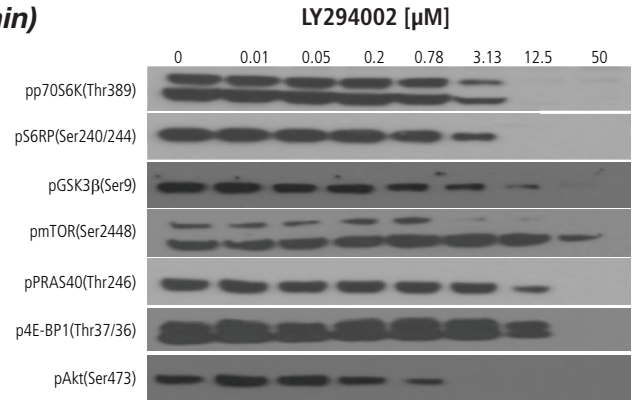
Recent advances in understanding the molecular pathogenesis of cancer present challenges and opportunities in oncology research. For example, somatic mutations that result in up-regulation of the PI3 kinase (PI3K) signaling pathway are common in cancers and numerous discovery programs seek to identify specific inhibitors. Discovery teams demand a rapid screening approach to accurately assign $IC_{50}$ and correctly rank order compounds with respect to their potency and in vivo efficacy. One research group replaced 6 weeks of western blot screening of 150 compounds with less than one week of testing using MSD multiplex assays. In just a few days, eight-point titrations of 150 compounds, in triplicate, against three phosphoproteins (10 800 data points) were completed.

> *"Compared to MSD assays, western blotting is time-consuming, with most antibodies requiring overnight incubations. Western blotting assays using two antibodies require 2 days, whereas the MSD assay can be completed and analyzed within 1 day."* (Gowan et al.)[1]

The figures below illustrate the dose-dependent effects of inhibitors of Akt signaling pathway using human MCF7 tumor cell lines stimulated with IGF-1. MCF7 cells were treated with the indicated amount of inhibitor followed by a 20 minute stimulation with IGF-1 (50 nM).

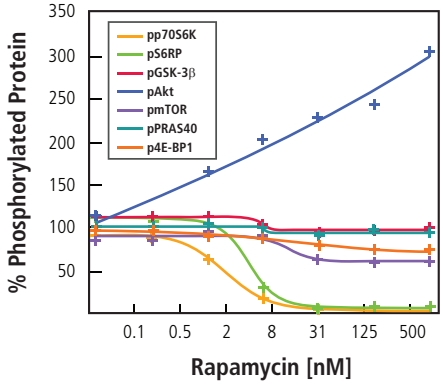
***LY294002 Titration (2 hr) + IGF-1 (50 nM; 20 min)***

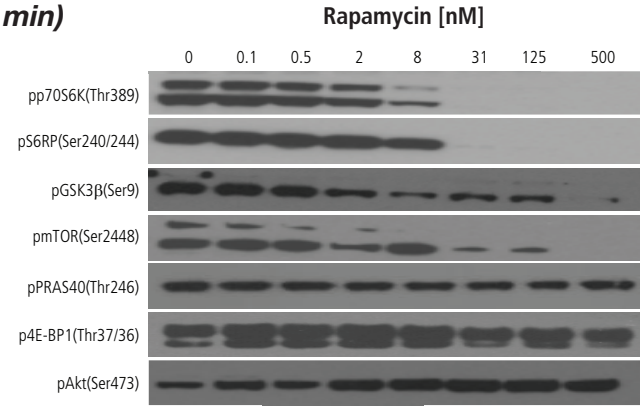



- **¼ of whole cell lysate preparation from a well of a 96-well plate is used for each MSD data point. A 2-fold larger amount of lysate was used to complete traditional western blot analysis.**

***Rapamycin Titration (2 hr) + IGF-1 (50 nM; 20 min)***




By using MSD's multiplexed assays containing targets of the PI3K pathway, Raynaud et al.[2] presented supportive data for the development of GDC-0941, a potent inhibitor of PI3K in glioblastoma cell line. GDC-0941 is now in clinical trials for solid breast tumor malignancies and non-Hodgkins lymphoma.

# The Transition from Discovery:

## Multiplex Assays for Pharmacodynamics in Human Xenografts

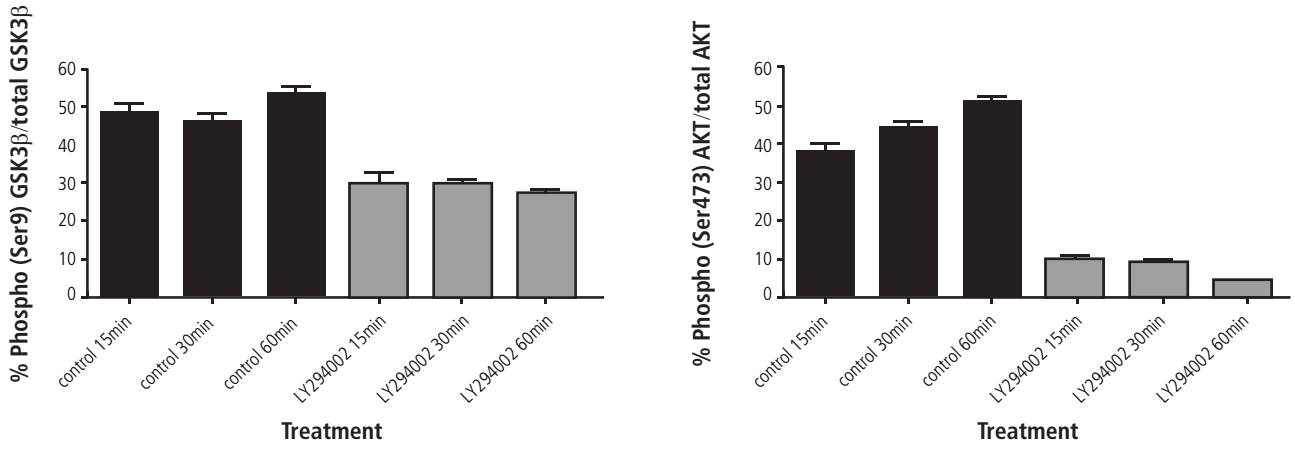
The same MSD assays that power compound characterization in tumor cell lines have proven sensitive and robust enough for the study of compound activity in xenografts. Gowan et al[1] selected MSD over all other available methods such as enzyme-linked immunosorbent assay (ELISA), western blotting, immunohistochemistry, reverse transcription-polymerase chain reaction, flow cytometry, magnetic resonance imaging/spectroscopy, and gene expression profiling. The MSD Phospho/Total Akt and Phospho/Total GSK-3β multiplex assays were used to measure both in vitro and in vivo changes in response to LY294002 within two different tumor types.

## Major Obstacles with Conventional Methods

- **Large sample volume**
- **Several days of procedure and analysis**
- **High matrix interference**
- **Qualitative versus quantitative**

## MSD Solutions and Customer's Comments

- **Reduced sample volume**
  *"…possibility of detailed and accurate analyses of valuable patient material as several proteins can be screened from a small sample within 1 day."*[1]
- **Rapid and robust**
  *"The assays are robust (coefficient of variation for phospho-Akt 13.4%) and offer significant advantages (in terms of speed and quantitation) over western blots."*[1]
- **Low background**
  *"The assay background signal…detected with MSD technology was very low…and the peak signal was very high… resulting in a superior signal-to-noise ratio…"*[3]
- **Matrix compatibility**
  *"MSD protein profiling system offers a sensitive, adaptable platform for sandwich immunoassays in microplates with one to 10 carbon electrode arrays per well."*[1]
- **Absolute quantification**
  *"…novel sandwich assay (ECL) as it provides sensitivity…accuracy, reproducibility and absolute quantification…"*[4]
- **One technology and one platform for different stages of drug discovery and development**
  *"This optimized procedure can be used for both in vitro and in vivo analysis, unlike an established fixed-cell ELISA with a time resolved fluorescent end point."*[1]




Inhibition of phosphorylation on Akt and GSK3β phosphoprotein in human tumor xenograft treated with LY294002. Approximately 3 X $10^6$ cells were injected bilaterally subcutaneously in the flanks of female NCr athymic mice. The effect of LY294002 at 100 mg/kg (two doses, 30 min apart) was compared to solvent controls at 15 min, 30 min, and 1 h after administration. Tumors were excised and immediately snapfrozen in liquid nitrogen. Samples were stored at –80°C until processed. Tumors were ground and lysed in the optimal lysis buffer (buffer B). Protein concentrations were measured, and samples (10 µg) were analyzed using MSD phosphoprotein assays.[1]

# Monitoring the Periphery:

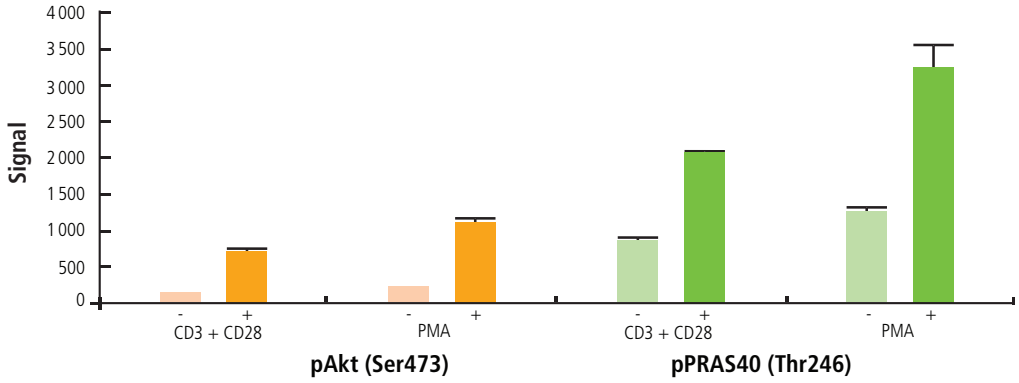
## Peripheral Blood Mononuclear Cells (PBMC) as Readout for Drug Efficacy

Chemical entities that inhibit kinases have been the subject of intense drug discovery and development owing to the documented role of these proteins in tumorigenesis. Pharmocodynamic studies ideally use the substrates of these kinases, phosphoproteins, to report drug activity. In many cases, the tumors that are the target of these therapies are unavailable or inaccessible. However, a number of the pathways that are the objective of therapeutic intervention are constitutively active in PBMCs. Tan et al.[5] have reviewed the concept of using peripheral cells to monitor drug activity.

The data below illustrate the utility of MSD phosphoprotein assays for monitoring drug activity against the PI3 kinase pathway using PBMCs.

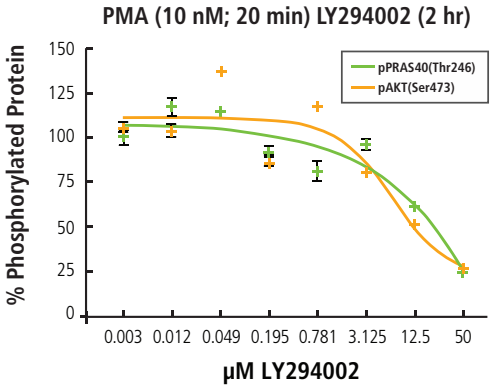
### Dose-dependent inhibition of Akt and PRAS40 in PBMCs with a potent PI3 Kinase inhibitor

Human PBMCs were purified from whole blood. Whole cell lysates were prepared and examined using the MSD MULTI-ARRAY Phospho-Akt (Ser473) and Phospho-PRAS40 (Thr246) Whole Cell Lysate Kits.

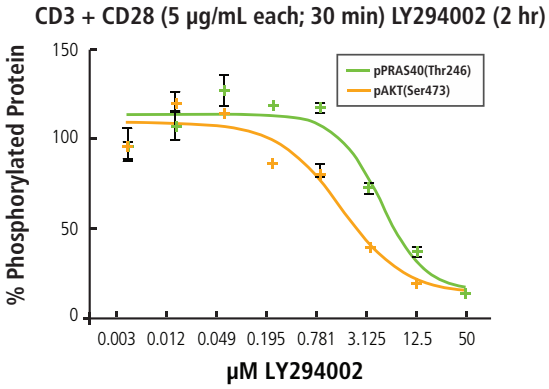



Human PBMCs were stimulated with either PMA (10 nM; 20 min) or CD3 + CD28 antibodies (5 µg/mL each; 30 min).

Human PBMCs were treated with serial dilutions of the PI3 Kinase inhibitor LY294002. The cells were then stimulated with either PMA (10 nM; 20 min) or CD3 + CD28 antibodies (5 µg/mL each; 30 min).




$IC_{50}$ curves for LY294002 inhibition of activated Akt and PRAS40 proteins after stimulation with PMA.




$IC_{50}$ curves for LY294002 inhibition of activated Akt and PRAS40 proteins after stimulation with a mixture of CD3 and CD28 antibodies.

> *"This technology…is cost-effective compared with western blots."* (Gowan et al.)[1]

MSD multiplex panels have also been used by Ghosh, et al.[6] to profile cytokine response in PBMCs treated with toll-like receptor agonists individually or in combination. The cell-free supernatants were collected and tested for IFN-α, IFN-β, IFN-γ, IL-12p70, and TNF-α using MULTI-SPOT plates on the MESO SCALE DISCOVERY platform.

# Recombinant Phosphoprotein Standards:

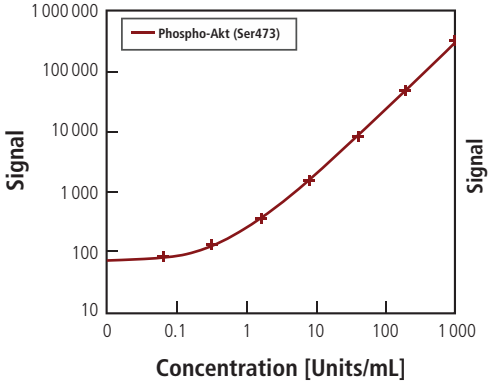
## Quantitative Assignment of Abundance

The absolute quantification of phosphoproteins in clinical samples will greatly add to their utility as prognostic, diagnostic, and pharmacodynamic markers. Such precision will require the use of purified, recombinant protein standards as calibrators, as is routinely practiced in concentration assignments for soluble proteins in bodily fluids. The production and handling of recombinant protein calibrators must assure that the epitopes of the capture and detection antibodies are readily recognized and that the post-translational phosphorylation is not lost. In the example below, MSD site-specific phosphorylated and total Akt recombinant standards are compared to MSD whole cell lysates and non-quantitative western blot analysis.

> *"…novel sandwich assay (ECL) as it provides sensitivity… accuracy, reproducibility and absolute quantification…"* (Cao et al.)[4]

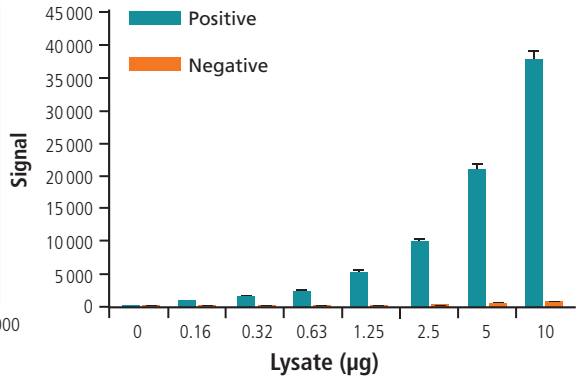
### *Phospho-Akt (Ser473) Assay*

**Calibrators**



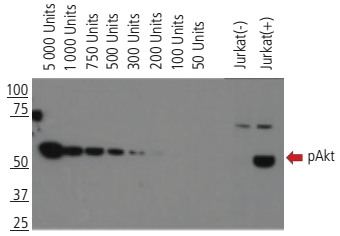

1 Unit = 100 pg of Total Akt recombinant protein used in the activation reaction

**Whole Cell Lysate**



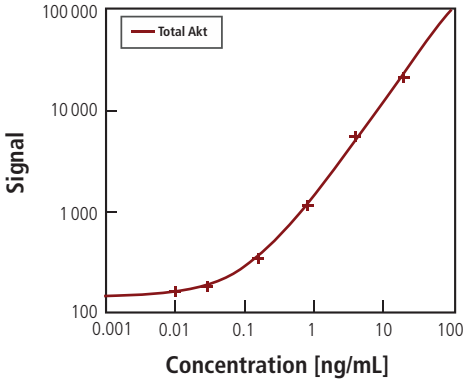

Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 µM, 2.25 hours) (negative).

**Western Blot**

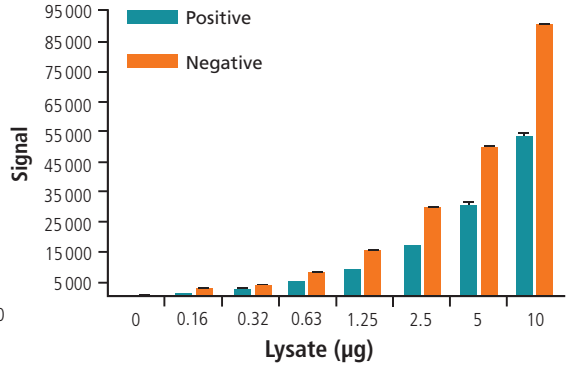



### *Total Akt Assay*

**Calibrators**

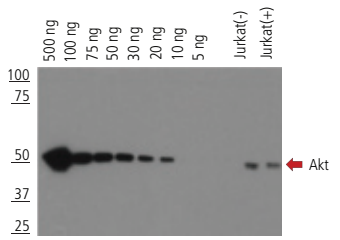



**Whole Cell Lysate**




Logarithmically growing Jurkat cells (positive) were treated with LY294002 (50 µM, 2.25 hours) (negative).

**Western Blot**



# Representative Cell Signaling Markers

## Singleplex Kits

| | |
|---|---|
| 4E-BP1 | *pT37/46*[§]<br>*Total*[‡] |
| Akt | *pS473*<br>*pT308*<br>*Total* |
| Aurora A | *pT288* |
| BAD | *pS112*<br>*Total* |
| c-Jun | *pS63* |
| c-Kit | *pY721*<br>*Total* |
| Caspase-3 | *p20/p17 (cleaved)*<br>*Total* |
| EGFR | *pY1173*<br>*pY1068*<br>*Total* |
| eIF2α | *pS51*[‡] |
| EphB4 | *Total*[‡] |
| ErbB2 | *pY1248*<br>*Total* |
| ErbB3 (HER3) | *pY*[‡] |
| ERK1/2 | *(pT202/pY204), (pT185/pY187)*<br>*Total* |
| ERK5 | *pT218/pY220* |
| FOXO3a | *pT32*[§] |
| FRS2 | *pY196*<br>*pY436* |
| GAPDH[‡] | |
| GSK-3α | *pS21*<br>*Total*[‡] |
| GSK-3β | *pS9*<br>*Total* |
| HIF-1α | *Total* |
| Histone H3 | *pS10* |
| HSP27 | *pS15*<br>*pS78*<br>*pS82*<br>*Total* |
| HSP70 | *Total* |
| IGF-1R | *pY*<br>*Total* |
| IR | *pY*<br>*Total* |
| IRS-1 | *pS312*<br>*Total* |
| JAK2 | *pY1007/1008*[‡]<br>*Total*[‡] |
| JNK | *pT183/pY185*<br>*Total* |
| MAPKAPK-2 | *pT334* |
| MEK1/2 | *pS217/221*<br>*Total* |
| MEK2 | *Total* |
| Met | *pY1349*<br>*Total* |
| mTOR | *pS2448* |
| NFκB | *pS536*<br>*pS468*<br>*Total*[‡] |
| p21 | *Total*[‡] |
| p38 | *pT180/pY182*<br>*Total* |
| p53 | *pS15*<br>*Total* |
| p70S6K | *pT421/pS424*<br>*pT389*<br>*Total* |
| PARP | *D214 (cleaved)* |
| PDGFR-β | *pY751* |
| PDH E1α | *pS293*[‡] |
| Pleckstrin | *pS*[‡] |
| PRAS40 | *pT246*<br>*Total*[§] |
| Rb | *pS608*<br>*pS780*<br>*Total* |
| RON | *pY1238/1239*[‡] |
| S6RP | *pS235/236*<br>*pS240/244*<br>*Total* |
| Smad1 | *pS463/465*[‡] |
| SRC3 | *Total*[‡] |
| STAT3 | *pY705* |
| STAT4 | *pY693*<br>*Total* |
| STAT5a,b | *pY694*<br>*Total* |
| Tau | *pT231*<br>*pS202*<br>*pS262*[‡]<br>*pS396*[‡]<br>*pT181*<br>*Total* |
| Tuberin | *pT1462*[‡] |
| VASP | *pS157*<br>*pS239*<br>*Total* |
| VEGFR2 | *pY1054*<br>*pY1175*<br>*Total* |

[‡]Available through our Prototype Printing Services [§]Coming Soon

## Multiplex Kits

### Activated/Total Panels

- Akt (pS473), Total Akt
- BAD (pS112), Total BAD
- Caspase-3 (Cl. p20/p17), Total Caspase-3
- EGFR (pY1173), Total EGFR
- eIF4E (pS209), Total[‡]
- ErbB2 (pY1248), Total ErbB2
- ERK1/2 (pT202/pY204)/(pT185/pY187), Total ERK1/2
- GSK-3α (pS21), Total GSK-3α[‡]
- GSK-3β (pS9), Total GSK-3β
- HSP27 (pS15), Total HSP27
- HSP27 (pS78), Total HSP27
- HSP27 (pS82), Total HSP27
- JNK (pT183/pY185), Total JNK
- c-Kit (pY721), Total c-Kit
- MEK1/2 (pS217/221), Total MEK1/2
- Met (pY1349), Total Met
- mTOR (pS2448), Total mTOR
- NFκB (pS468), Total NFκB[‡]
- NFκB (pS536), Total NFκB[‡]
- p38 (pT180/pY182), Total p38
- p53 (pS15), Total p53
- p70S6K (pT421/pS424), Total p70S6K
- Rb (pS608), Total Rb
- Rb (pS780), Total Rb
- S6RP (pS240/244), Total S6RP
- STAT5a,b (pY694), Total STAT5a,b
- Tau (pT231), Total Tau
- VASP (pS157), VASP (pS239), Total VASP

## Multiplex Kits

- MAP Kinase Panel (Phosphoprotein) (pERK1/2, pJNK, pp38)
- MAP Kinase Panel (Total Protein) (ERK1/2, JNK, p38)
- EGFR Family Panel (pEGFR, pErbB2, pIGF-1R)
- EGFR, ErbB2, ErbB3 (pY detection)[‡]
- Akt Signaling Panel (Phosphoprotein) (pAkt, pp70S6K, pGSK-3β)
- Akt Signaling Panel (Total Protein) (Akt, p70S6K, GSK-3β)
- Akt Signaling Panel II (Phosphoprotein) (pAkt, pp70S6K, pGSK-3β, pS6RP)
- Akt Signaling Panel II (Total Protein) (Akt, p70S6K, GSK-3β, S6RP)[‡]
- Apoptosis Panel (pp53, p53, Cl. Caspase-3, Cl. PARP)
- ERK-STAT3 Cascade Panel (pERK1/2, pMEK1/2, pSTAT3)
- Insulin Signaling Panel (Phosphoprotein) (pIR, pIGF-1R, pIRS-1)
- Insulin Signaling Panel (Total Protein) (IR, IGF-1R, IRS-1)
- STAT Panel (pSTAT3, pSTAT4, pSTAT5a,b)

## Ubiquitin Pathway Kits

- MDM2 - p53 complex
- Total MDM2
- Ubiquitinated MDM2
- Total p53
- Ubiquitinated p53
- Self-Ubiquitinated MDM2 (Activity Assay)

### Multiplex Panels

- MDM2 (Ub), Total MDM2
- p53 (Ub), Total p53

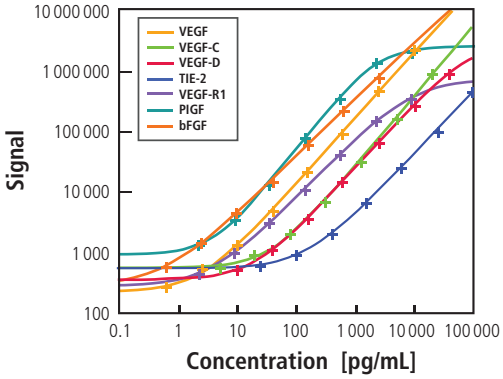
# Inflammation and Cancer:

## Evaluation of Angiogenic Biomarkers

**Angiogenesis**, the growth of new blood vessels, has emerged as a fundamental step in tumor growth, metastatic progression, and results in poor patient outcomes in several disease states including cancer. Understanding the critical regulators and pathways involved in the formation of new blood vessels is a complex process that involves not only neoplastic cells, but also endothelial cells, the basal membranes of neighboring capillaries and the stroma of the growing tumor mass. Key angiogenic factors, most notably VEGF, may serve as potential prognostic markers for disease activity or tumor progression and are often the target of inhibitors, receptor blockades or other chemotherapeutics.

MSD offers a variety of assays for pro-angiogenic and related proteins that provide a comprehensive assessment and understanding of the endothelial, vascular, and tumor cell microenvironment.

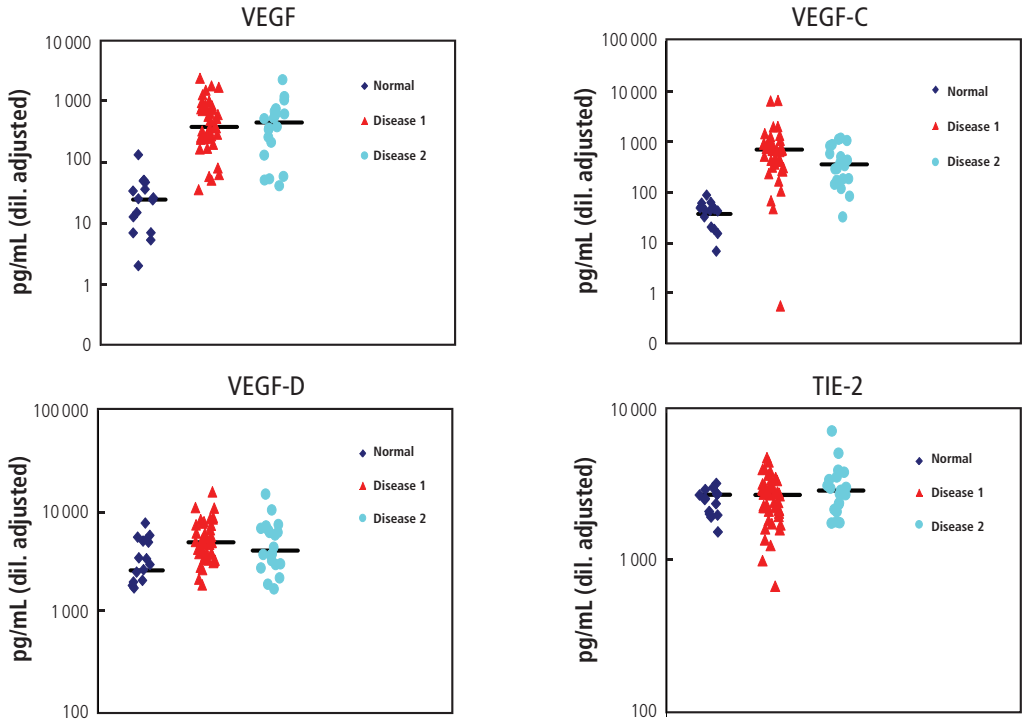
### *Human Angiogenesis Panel 1*




| | Analyte | | | | | | |
|---|---|---|---|---|---|---|---|
| | VEGF | VEGF-C | VEGF-D | TIE-2 | VEGF-R1 | PlGF | bFGF |
| LLOD (pg/mL) | 0.64 | 5.4 | 2.4 | 26 | 0.42 | 0.35 | 0.10 |

LLOD (Lower Limit of Detection) is defined as 2.5 SD above the background.

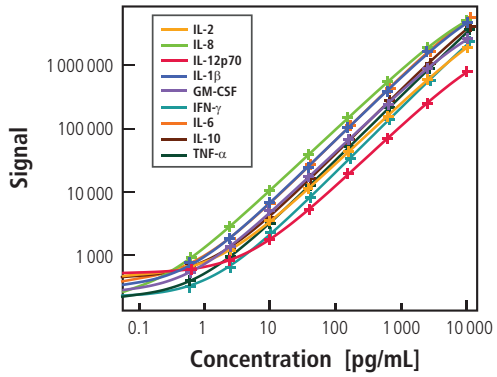
Illustrated below are biomarker levels in sera from normal and diseased populations tested at 2-fold dilution on the MSD Angiogenesis Panel 1. Some biomarkers may be elevated or suppressed in a given disease state whereas other biomarkers are not effected.



# Biomarker Profiling:
## Analysis of Clinical Samples

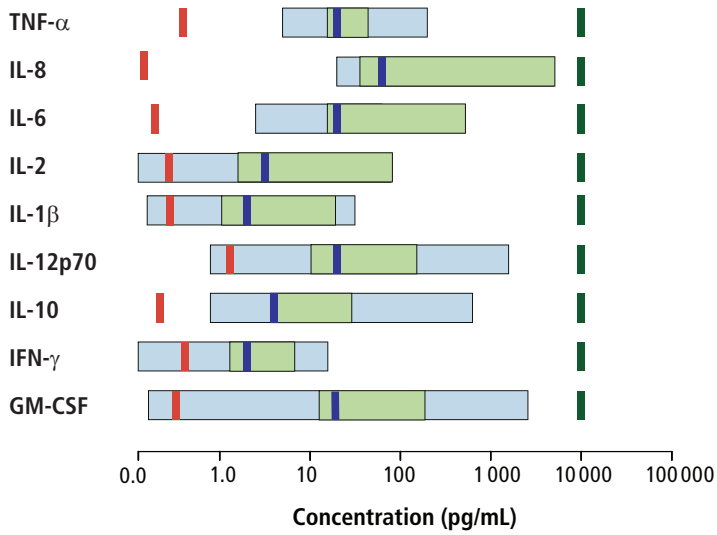
Clinical samples push the limits of traditional methods for immunoassays (e.g. ELISA, RIA). Complex matrices (e.g. sputum, vaginal fluids, etc.), widely ranging concentations of analytes, and limited sample volume can make assays intractable. MSD's assays improve sensitivity, expand the dynamic range, enable measurement of multiple analytes from a single sample (i.e. multiplexing), and work well in difficult sample matrices. The MSD platform has also proven to be well-suited for use in validated work environments with available packages for IQ/OQ/PQ and software designed to support 21 CFR Part 11 and GLP compliance.

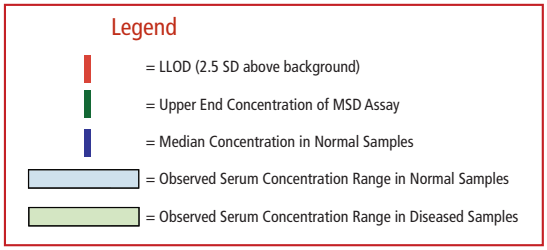
### *Representative Data from Clinical Samples using MSD Human ProInflammatory 9-Plex*




| | Analyte | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | IL-2 | IL-8 | IL-12p70 | IL-1β | GM-CSF | IFN-γ | IL-6 | IL-10 | TNF-α |
| LLOD (pg/mL) | 0.35 | 0.09 | 1.4 | 0.36 | 0.34 | 0.53 | 0.27 | 0.21 | 0.50 |

LLOD (Lower Limit of Detection) is defined as 2.5 SD above the background.




In the above study*, a total of 127 human serum samples were tested, which included diseased pools and controls. The upper end of the calibrator curve for this panel is 10 000 pg/mL for all cytokines and the lower limit of detection (LLOD) was determined as 2.5 standard deviations above the background.

The MSD MULTI-ARRAY platform has been used by Bafadhel, et al.[8] to study a panel of 11 cytokines and chemokines in a complex matrix such as sputum samples from subjects with chronic obstructive pulmonary disorder (COPD). The rapid assays enabled the measurement of multiple cytokines from several samples with minimal dilution and indicated that IL-5 in sputum is a repeatable and reproducible measurement of COPD.

> *"Reproducible interlaboratory measurement of all cytokines was found only for the MSD assays."* (Fichorova et al.)[9]

* The Biomarker Reference Set for Cancers in Women (BRSCW) was provided by the National Cancer Institute on behalf of the Early Detection Research Network (EDRN).

# Representative Cytokines and Other Oncology Markers

## Human

BDNF
CTACK‡
Eotaxin
Eotaxin-3
G-CSF
GM-CSF
ENA-78‡
GRO-α
IFN-α2a
IFN-β
IFN-γ
IL-1α
IL-1β
IL-1ra‡
IL-2
IL-4
IL-5
IL-6
IL-6R
IL-7
IL-8
IL-10
IL-12
IL-12p40
IL-12p70
IL-13
IL-15
IL-16
IL-17
IL-18‡
IL-23‡
IP-10
I-TAC
M-CSF
MCP-1
MCP-2‡
MCP-3‡
MCP-4
MDC
MIG
MIP-1α
MIP-1β
MIP-3α
MIP-3β‡
MMP-1
MMP-2
MMP-3
MMP-8‡
MMP-9
MMP-10
NGAL‡
RANTES
SDF-1α‡
TARC
TGF-β1
TGF-β2‡
TGF-β3‡
TIMP-1*
TIMP-2‡
TNF-α
TNF-RI
TNF-RII
TSLP‡
TWEAK‡

## Mouse

GM-CSF
IFN-γ
IL-1β
IL-2
IL-4
IL-5
IL-6
IL-10
IL-12
IL-12p40
IL-12p70
IL-13
IL-17
KC/GRO/CINC (CXCL1)
MCP-1
M-CSF‡
MIP-1α‡
MIP-1β‡
MIP-2‡
MIP-3α‡
MMP-9‡
RANTES
TNF-α
TNF-RI
TNF-RII

## Rat

CINC-2‡
CINC-3‡
CNTF-1‡
GM-CSF
IFN-γ
IL-1α
IL-1β
IL-2‡
IL-4
IL-5
IL-6
IL-10‡
IL-13
KC/GRO/CINC (CXCL1)
MCP-1
MIP-3α
TIMP-1‡
TNF-α

## Canine

GM-CSF‡
IFN-γ‡
IL-2
IL-6
IL-8
IL-10
MCP-1‡
TNF-α

‡Available through our Prototype Printing Services *Qualified Kit

## Multiplex Panels

| Panel | Analytes |
|---|---|
| Human TH1/TH2 7-Plex | IFN-γ, IL-2, IL-4, IL-5, IL-10, IL-12p70, IL-13 |
| Human TH1/TH2 10-Plex | IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12p70, IL-13, TNF-α |
| Human ProInflammatory 4-Plex I | IFN-γ, IL-1β, IL-6, TNF-α |
| Human ProInflammatory 4-Plex II | IL-1β, IL-6, IL-8, TNF-α |
| Human ProInflammatory 7-Plex | IFN-γ, IL-1β, IL-6, IL-8, IL-10, IL-12p70, TNF-α |
| Human ProInflammatory 9-Plex | GM-CSF, IFN-γ, IL-1β, IL-2, IL-6, IL-8, IL-10, IL-12p70, TNF-α |
| Human MMP 2-Plex | MMP-2, MMP-10 |
| Human MMP 3-Plex | MMP-1, MMP-3, MMP-9 |
| Human Chemokine 7-Plex | Eotaxin, IL-8, IP-10, MCP-1, MCP-4, MIP-1β, TARC |

| Panel | Analytes |
|---|---|
| Human Chemokine 9-Plex | Eotaxin, Eotaxin-3, IL-8, IP-10, MCP-1, MCP-4, MDC, MIP-1β, TARC |
| Human Demonstration 4-Plex | IL-1β, IL-2, IL-6, TNF-α |
| Human Demonstration 7-Plex | GM-CSF, IL-1β, IL-2, IL-5, IL-6, IL-8, TNF-α |
| Human Demonstration 10-Plex | GM-CSF, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12p70, TNF-α |
| Mouse TH1/TH2 9-Plex | IFN-γ, IL-1β, IL-2, IL-4, IL-5, KC/GRO/CINC, IL-10, IL-12 total, TNF-α |
| Mouse ProInflammatory 7-Plex | IFN-γ, IL-1β, IL-6, IL-10, IL-12p70, KC/GRO/CINC, TNF-α |
| Rat Demonstration 7-Plex | IFN-γ, IL-1β, IL-4, IL-5, IL-13, KC/GRO/CINC, TNF-α |
| Canine ProInflammatory Panel 3 | IL-2, IL-6, IL-8, TNF-α |

## Oncology & Vascular Markers

AFP (human)
Angiopoietin 1 (human)‡
Angiopoietin 2 (human)‡
E-Cadherin (human)
P-Cadherin (human)
Cancer Antigen 125 (human)
Cancer Antigen 19.9 (human)
Cancer Antigen 15.3 (human)‡
CEA (human)
c-Kit (human)
CRP (human)
EGF (human)‡
sEGFR (human)
Endoglin (human)‡
EPO (human, mouse/rat)
sErbB2 (human)
sFas (human)‡
FGF acidic (human)‡
bFGF (human)
sFlt-1 (sVEGFR1) (human)
HGF (human)
HIF-1α (human)
IGFBP-1 (human)
sICAM-1 (human)
sICAM-3 (human)
KDR (sVEGFR2) (human)
sMet (human)‡
MMP-9 (human)
β-NGF (human)‡
Osteopontin (human)
p21 (human)‡
uPA (human)‡
PEDF/Serpin F1 (human)‡
PIGF (human)
PSA (human)‡
SAA (human)
E-Selectin (human)
L-Selectin (rat)‡
P-Selectin (human)
TGF-β1 (human)
TGF-β2 (human)‡
TGF-β3 (human)‡
TIMP-1 (human*, rat‡)
Thrombomodulin (human)
sVCAM-1 (human)
$VEGF_{(121/165)}$ (human, mouse/rat)
VEGF-C (human)‡
VEGF-D (human)‡

### Multiplex Panels

Acute Phase Protein Panel 1 (rat) (A2M, AGP)*
c-Kit (pY721), Total c-Kit (human)
EPO, VEGF (human)
Growth Factor Panel I (human) (bFGF, VEGF, sFlt-1, PIGF)
Growth Factor Panel II (human) (c-Kit, KDR)
Hypoxia Panel (mouse/rat) (EPO, VEGF)
Hypoxia Panel (human) (EPO, IGFBP-1, VEGF)
Vascular Injury Panel I (human) (sICAM-3, E-Selectin, P-Selectin, Thrombomodulin)
Vascular Injury Panel II (human) (CRP, sICAM-1, sVCAM-1, SAA)

For a complete list of products please check our website at www.mesoscale.com

# Prototype Printing Services

MSD offers Prototype Printing Services to facilitate assay development by customers. Prototype Printing Services provide the customer with a rapid and convenient way to get MSD plates coated with materials of their choice.

**How to Order**

**STEP 1:** Obtain a quote from MSD for your analyte of interest by contacting MSD Customer Service

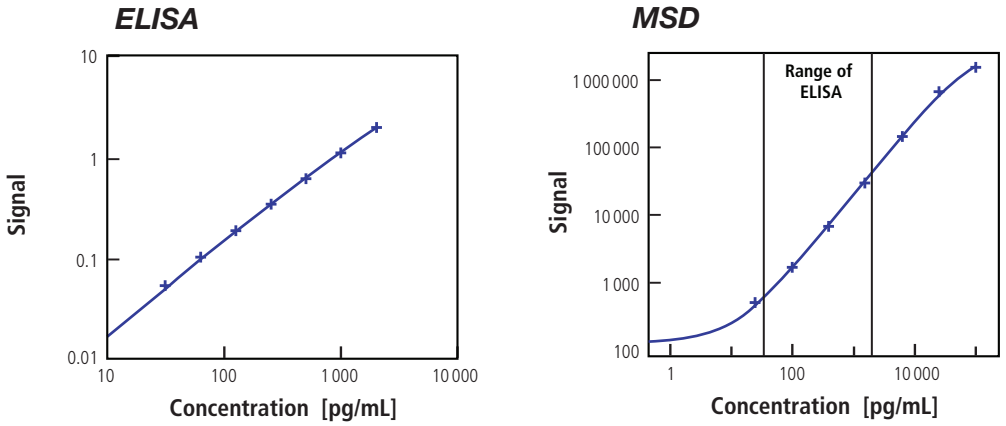
**STEP 2:** Place your order
Reference the quote number on your order.

**STEP 3:** Receive your Prototype assay

## Types of Capture Materials coated on MSD plates

### 1. Antibodies

Antibodies are readily coated on MSD plates. Coating antibodies expedites the conversion of ELISAs to the MSD platform with ease. An example of conversion of an ELISA to an MSD assay is demonstrated here. Customers routinely see significant improvements in assay performance when ELISAs are converted to the MSD format.




### 2. Proteins and Peptides

The MSD platform is very effective for screening antibodies. By testing antibodies against multiple antigens, customers can test for cross-reactivity and specificity in the same well. The example shown here demonstrates the use of an MSD 10-Spot plate for antibody screening. Titration of hybridoma supernatants identifies the high binding clones, which are subsequently tested for cross-reactivity as well as specificity.


MSD 10 Spot Plate Coated with Different Proteins (antigens)

Titration and Screening of hybridoma on MSD plate

Cross-reactivity and Specificity Testing of Clones

### 3. Carbohydrates and Polysaccharides

Carbohydrates have been successfully coated on MSD plates, which has enabled the testing of different Pneumococcal vaccines by Marchese, et al.[10] MSD plates coated with lipopolysaccharides have been used by Thompson, et al.[11] for serodiagnosis of Brucellosis in ruminants. Other than the above mentioned materials, MSD plates are highly amenable to coating with viral proteins, cell lysates, etc.

## MSD's Prototype Printing Services Offer:

**1. ELISA Conversion Packages:** MSD offers conversion packages for assay development.

**2. Coating Optimization Package:** MSD can provide plates manufactured using several coating conditions on different plate surfaces as a means of determining the optimal conditions for a given assay.

**3. On-site Scientific Support:** Support for assay development can be obtained from our Field Application Scientists and the Scientific Support team (ScientificSupport@mesoscale.com or 301-947-2025).

**4. MSD Early Access Assays:** These are assays for which MSD has identified a working antibody pair and calibrator. MSD can provide suggestions for assay diluents and protocol.

# References


[1] Gowan, S.M., Hardcastle, A., Hallsworth, A.E., Valenti, M.R., Hunter, L.J., de Haven Brandon, A.K., Garrett, M.D., Raynaud, F., Workman, P., Aherne, W., Eccles, S.A. ***Application of Meso Scale Technology for the Measurement of Phosphoproteins in Human Tumor Xenografts.*** Assay and Drug Dev Technol. 2007 Jun; Vol. 5 (3):391-401.

[2] Raynaud, F.I., Eccles, S.A., Patel, S., Alix, S., Box, G., Chuckowree, I., Folkes, A., Gowan, S., De Haven Brandon, A., Di Stefano, F., Hayes, A., Henley, A.T., Lensun, L., Pergl-Wilson, G., Robson, A., Saghir, N., Zhyvoloup, A., McDonald, E., Sheldrake, P., Shuttleworth, S., Valenti, M., Wan, N.C., Clarke, P.A., Workman, P. ***Biological properties of potent inhibitors of class I phosphatidylinositide 3-kinases: from PI-103 through PI-540, PI-620 to the oral agent GDC-0941.*** Mol Cancer Ther. 2009 Jul; Vol. 8(7):1725-1738.

[3] Liang, M., Klakamp S.L., Funelas, C., Lu, H., Lam, B., Herl, C., Umble, A., Drake, A.W., Pak, M., Ageyeva, N., Pasumarthi, R., Roskos, L. ***Detection of High- and Low-Affinity Antibodies Against a Human Monoclonal Antibody Using Various Technology Platforms.*** Assay and Drug Dev Technol. 2007 Oct; Vol. 5(5): 1-8.

[4] Cao, L., Yu, Y., Darko, I., Currier, D., Mayeenuddin, L.H., Wan, X., Khanna, C., Helman, L.J. ***Addiction to Elevated Insulin-like Growth Factor I Receptor and Initial Modulation of the AKT Pathway Define the Responsiveness of Rhabdomyosarcoma to the Targeting Antibody.*** Cancer Research. 2008 Oct; Vol. 68:8039-8048.

[5] Tan, D.S.W.., Thomas, G.V., Garrett, M.D., Banerji, U., De Bono, J.S., Kaye, S.B., Workman, P., ***Biomarker-Driven Early Clinical Trials in Oncology A Paradigm Shift in Drug Development. The Cancer Journal.*** 2009 Sep-Oct; Vol. 15, Number 5.

[6] Ghosh, T.K., Michelson, D.J., Solberg, J.C., Lipson, K.E., Inglefield, J.R., Alkan, S.S. ***TLR-TLR cross talk in human PBMC resulting in synergistic and antagonistic regulation of type-1 and 2 interferons, IL-12 and TNF-alpha.*** Int Immunopharmacol. 2007 Aug; Vol. 7(8):1111-21.

[7] Kelly, R.J., Rajan, A., Force, J., Keen, C., Cao, L., Yu, Y., Choyke, P.L., Turkbey, B., Raffeld, M., Xi, L., Steinberg, S.M., Wright, J.J., Lopez-Chavez, A., Kummar, S., Guttierrez, M., Giaccone, G. ***Evaluation of KRAS mutations, angiogenic biomarkers and DCE-MRI in patients with advanced non-small cell lung cancer receiving sorafenib.*** Clin Cancer Res. 2011 Mar 1;17(5):1190-9.

[8] Bafadhel, M., Saha, S., Siva, R., McCormick, M., Monteiro, W., Rugman, P., Dodson, P., Pavord, I.D., Newbold, P., Brightling, C.E. ***Sputum IL-5 Concentration Is Associated with a Sputum Eosinophilia and Attenuated by Corticosteroid Therapy in COPD.*** Respiration. 2009; Vol. 78:256-262.

[9] Fichorova R.N., Richardson-Harman, N., Alfano, M., Belec, L., Carbonneil, C., Chen, S., Cosentino, L., Curtis, K., Dezzutti, C.S., Donoval, B. Doncel, G.F., Donaghay, M., Grivel, J.C., Guzman, E., Hayes, M., Herold, B, Hillier, S., Lackman-Smith, C., Landay, A., Margolis, L., Mayer, K.H., Pasicznyk, J.M., Pallansch-Cokonis, M., Poli, G., Reichelderfer, P., Roberts, P., Rodriguez, I., Saidi, H., Sassi, R.R., Shattock, R., Cummins, J.E. Jr. ***Biological and technical variables affecting immunoassay recovery of cytokines from human serum and simulated vaginal fluid: a multicenter study.*** Anal Chem. 2008 Jun; Vol. 80(12): 4741-51.

[10] Marchese, R.D., Puchalski, D., Miller, P., Antonello, J., Hammond, O., Green, T., Rubinstein, L.J., Caulfield, M.J., Sikkema, D. ***Optimization and validation of a multiplex, electrochemiluminescence-based detection assay for the quantitation of immunoglobulin g serotype-specific antipneumococcal antibodies in human serum.*** Clin Vaccine Immunol. 2009 Mar; Vol. 16 (3):387-96.

[11] Thompson, W.L., Karpus W.J., Van Eldik, L.J. ***MCP-1-deficient mice show reduced neuroinflammatory responses and increased peripheral inflammatory responses to peripheral endotoxin insult. J Neuroinflam.*** 2008 Aug; Vol. 5:35.


References using MSD MULTI-ARRAY technology

# The MSD Advantage

## Assay Solutions and Services

- Over 300 single analyte kits
- Over 200 multiple analyte kits
- Customized multiplexed kits
- ELISA conversion packs
- QUICKPLEX® packs
- Prototype Printing Services
- On-site scientific support
- Contract assay development

## SECTOR® Instrumens

- Ultra-fast read time
- No fluidics
- No user calibration required
- Reliable measurements
- Integrated data analysis tool
- Comprehensive validation packages
- Software support for 21 CFR Part 11 compliance
- Comprehensive service plans

### Ordering Information

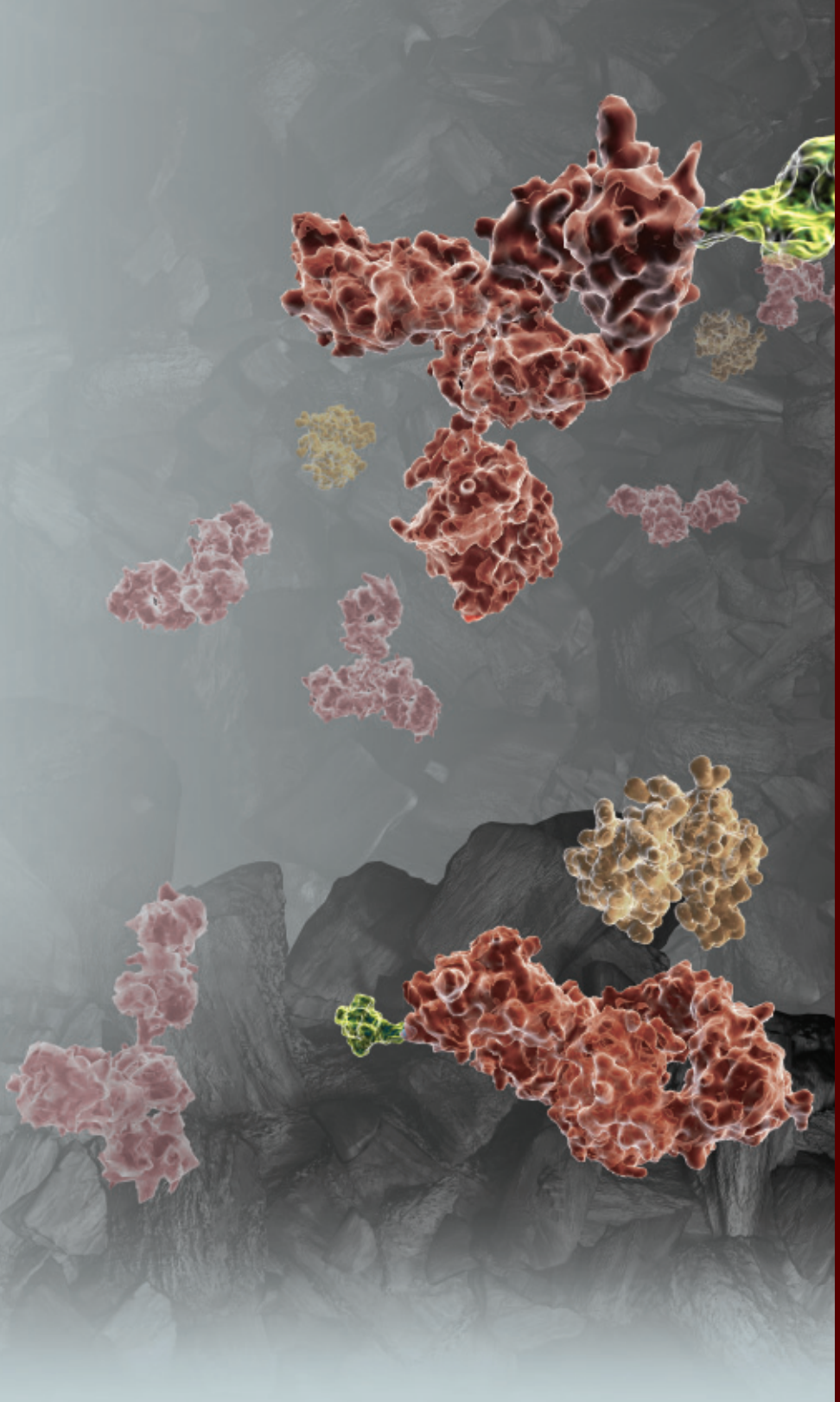
Purchase orders can be telephoned, faxed or emailed to the following:


TEL: 1.301.947.2085
FAX: 1.301.990.2776
EMAIL: customerservice@mesoscale.com


For a complete listing of our products, visit our online catalog at
www.mesoscale.com®

# ONCOLOGY APPLICATIONS

- Alzheimer's Disease
- BioProcess
- Cardiac
- Cell Signaling
- Clinical Immunology
- Cytokines
- Hypoxia
- Immunogenicity
- Inflammation
- Metabolic
- **Oncology**
- Toxicology
- Vascular



9238 Gaither Road Gaithersburg, MD 20877 USA Phone: 1.301.947.2085 Fax: 1.301.990.2776 Website: www.mesoscale.com


19505-v4-2011Jul

MESO SCALE DIAGNOSTICS®