MAP Kinase Activity Assays on MSD Multi-Array™ Platform

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Abstract

Mitogen-Activated Protein Kinases (MAPKs) are a widely conserved family of Serine/Threonine protein kinases involved in many cellular programs such as cell proliferation, cell differentiation, cell movement and cell death. MAPK signaling cascades are organized into three-tiered modules. MAPKs are phosphorylated and activated by MAPK-kinases (MAPKK), which in turn are phosphorylated and activated by MAPKK-kinases (MAPKKKs). The MAPKKKs are in turn activated by interaction with a family of small GTPases and/or other protein kinases connecting the MAPK module to the cell surface receptor or external stimulus.

We demonstrate a multiplexed assay approach for monitoring the activity of the entire MAPK cascade or any part of it using the Meso Scale Discovery Multi-Array™ platform. Whole proteins or short synthetic peptides can be employed as substrates. The assay protocols are compatible with high-throughput screening (HTS) and provide sensitive detection of enzyme activity across the MAP Kinase pathways.

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❷ Meso Scale Discovery Multi-Array Technology

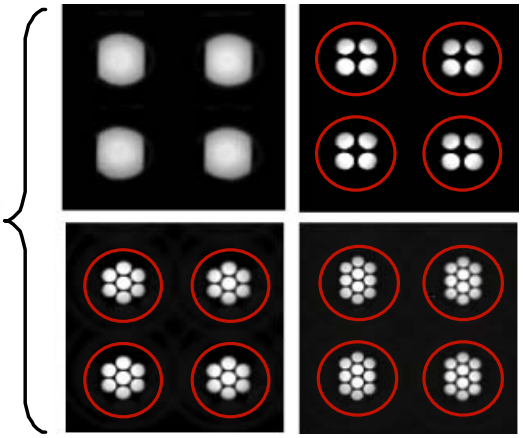
Instrument Features

- Highly sensitive
- SECTOR™ Imager 6000 designed for high-throughput screening (HTS)
- SECTOR™ PR 100 Reader ideal for assay development
- Custom optics
- High-speed motion control systems
- Electrochemiluminescence (ECL) detection

SECTOR™ Imager 6000

SECTOR™ PR 100 Reader

Plate Features



- Disposable Plates
- Carbon Electrodes with high binding capacity
- Suitable electrochemistry for ECL
- Biocompatible: direct immobilization of avidin, IgG, membrane fragments, intact cells, etc.
- Functional Assays: simple binding reactions, GPCRs, enzyme cascades, post-translational modification, etc.

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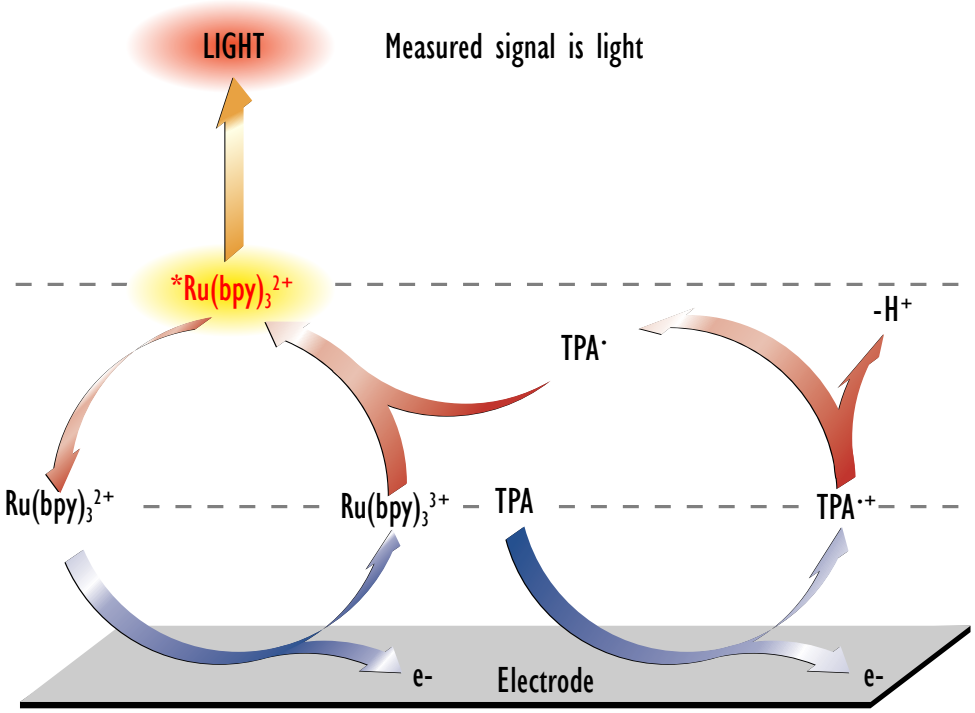
❸ Electrochemiluminescence (ECL)

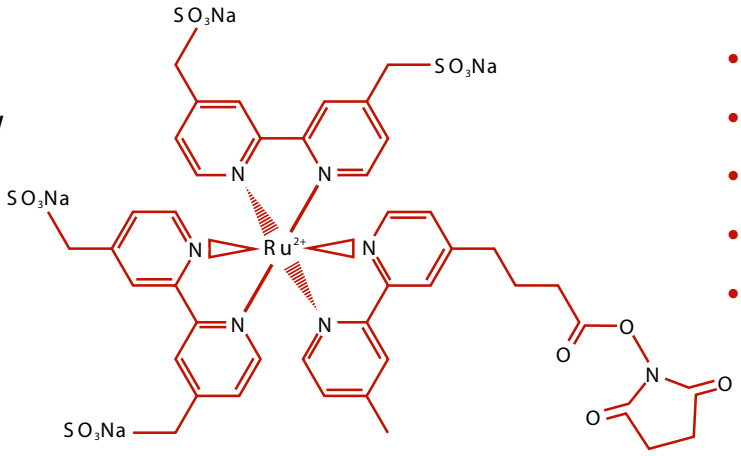


Luminescence
Emitting Light

Chemi-
Chemical Energy

Electro-
Electrochemically Initiated

MSD Sulfo-TAG™ Label



- Selective
- Convenient chemistry
- Robust, stable
- Few interferences

- Size, MW: ~1200 daltons
- Stability: Years
- Solubility: Aqueous, DMSO
- Functionality: Hydrophilic
- Specificity: High

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4 MAP Kinase Cascade

Example of MAPK Cascade

Activation Loop Sequence	xxxSMANSxxx	xxxTEYxxx	xxxPxTPxxx

Substrates Used for MAP Kinase Cascade Signaling

Whole Proteins:	Surface-bound Myelin Basic Protein (MBP), whole ERK and MEK molecules
Synthetic peptides:	Biotinylated short peptides, which contain xxxPxTPxxx, xxxTEYxxx or xxxSMANSxxx motifs, which mimic the target sequences of MBP, ERK and MEK respectively

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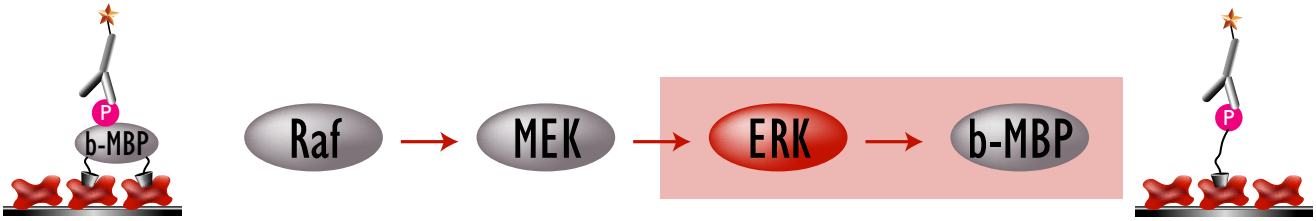
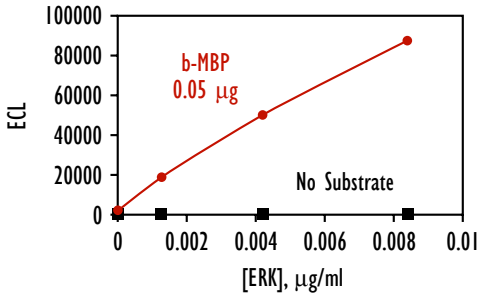
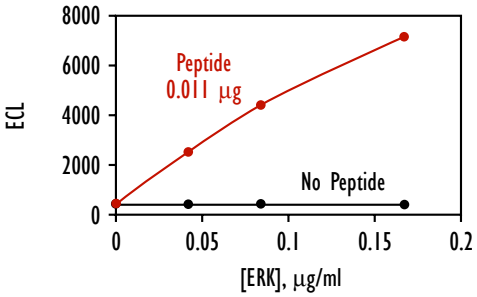
❺ ERK Activity Assays







Enzyme: Active ERK-2

Substrates: Biotinylated-MBP or biotinylated synthetic peptide containing PRTP motif immobilized on the surface of MSD Multi-Array 96-well plates coated with streptavidin

Antibodies: Mouse anti-phospho-MBP IgG (primary) and MSD Sulfo-TAG™-labeled anti-mouse IgG (secondary)

- Assay measures the activity of ERK kinase
- Linear response to ERK concentration
- Low background in the absence of ERK
- Signal-to-background ratio is ~45 at 0.001 µg/ml of ERK, with MBP used as the substrate
- Signal-to-background ratio is ~14 at 0.1 µg/ml of ERK, with a short peptide substrate

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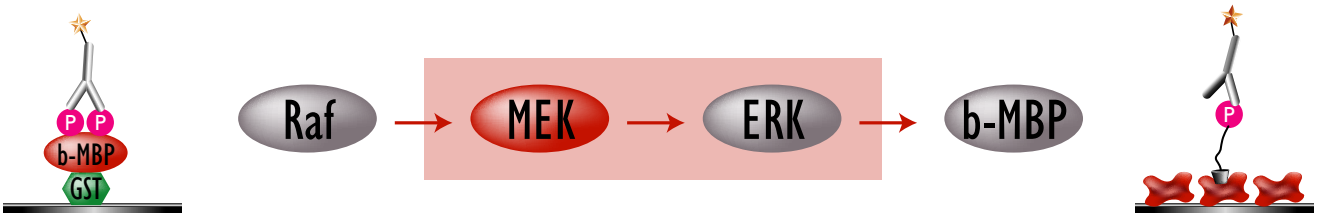
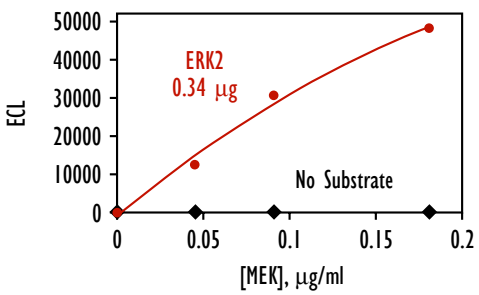
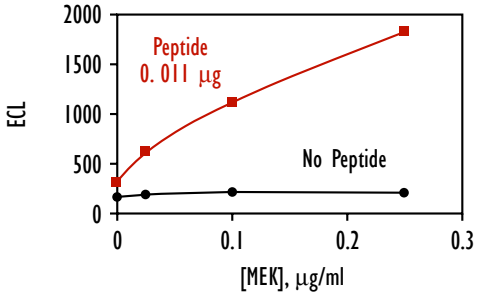
6 MEK Activity Assays







Enzyme: Active MEK-1

Substrates: Inactive GST-ERK-2 or biotinylated synthetic peptide containing SMANS motif immobilized on MSD Multi-Array 96-well plates coated with streptavidin

Antibodies: Mp44 Mouse IgG (primary) with MSD Sulfo-TAG-labeled anti-mouse IgG (secondary) or Rp44 Rabbit IgG (primary) with MSD Sulfo-TAG-labeled anti-rabbit IgG (secondary)

- Assay measures the activity of MEK kinase
- Approx. linear response to MEK concentration
- Low background in absence of MEK
- Signal-to-background ratio at 0.1 µg/ml of MEK is ~80 for whole ERK protein and ~5 for the peptide substrate

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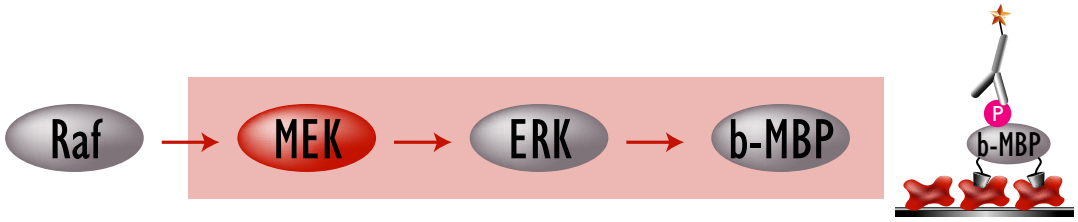
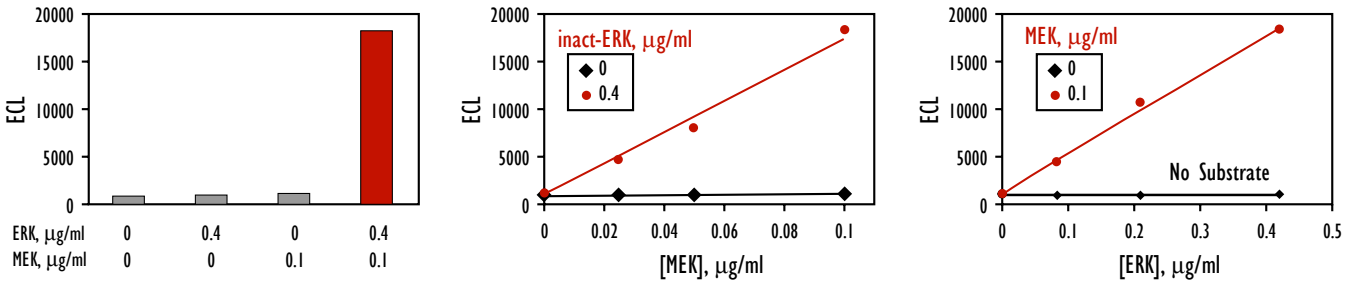
❼ Combined MEK & ERK Activity Assay





Enzyme: Active MEK-1, inactive ERK-2

Substrates: Biotinylated-MBP immobilized on MSD Multi-Array 96-well plates coated with streptavidin

Antibodies: Mouse anti-phospho-MBP IgG (primary), MSD Sulfo-TAG-labeled anti-mouse IgG (secondary)

- Assay measures the activity of MEK and ERK kinases simultaneously
- Linear response to both MEK and ERK
- Low background in absence of either enzyme
- Signal-to-background ratio is ~20 at 0.4 µg/ml ERK and 0.1 µg/ml MEK

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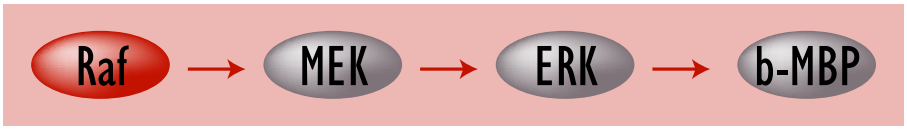
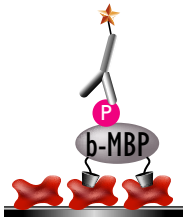
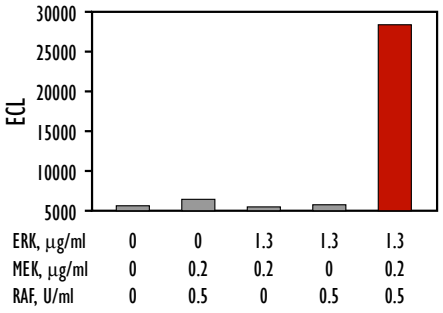
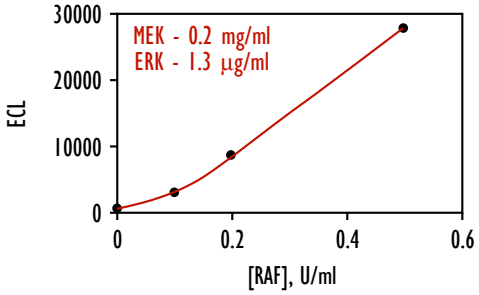
8 Assay for Entire MAPK Cascade









Enzyme: Active Raf-1; inactive MEK-1 and ERK-2

Substrates: Biotinylated-MBP immobilized on MSD Multi-Array 96-well plates coated with streptavidin

Antibodies: Mouse anti-phospho-MBP IgG (primary), MSD Sulfo-TAG-labeled anti-mouse IgG (secondary)

- Assay simultaneously monitors the activity of entire 3-step cascade
- Linear range exists for all 3 enzymes
- Low background in absence of any components of the MAPK cascade

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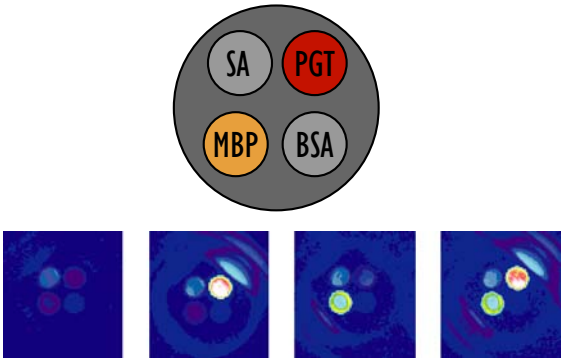
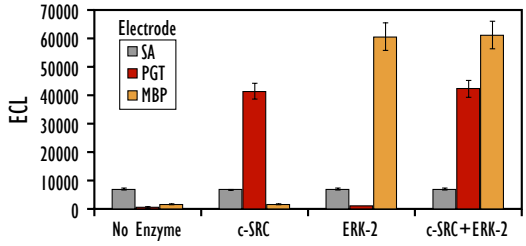
❾ Multiplexing: Serine/Threonine and Tyrosine Kinases in one well

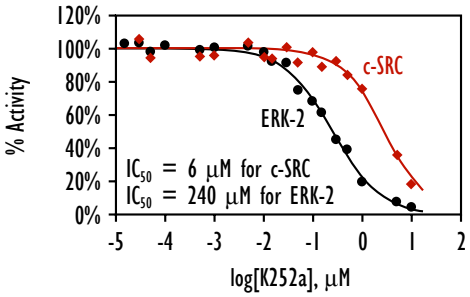
Enzyme: Active ERK-2 and Active c-SRC

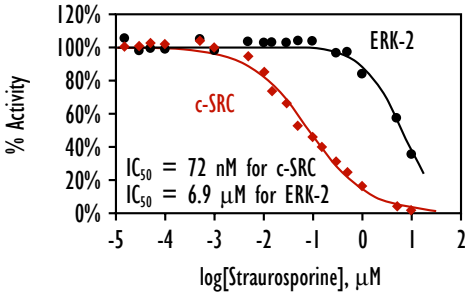
Substrates: poly-Glu-Tyr (PGT) and MBP immobilized on the surface of MSD Multi-Spot™ 4-spot plate

Antibodies: Mouse anti-phospho-MBP IgG, Mouse pY-20, Sulfo-TAG-labeled Anti-Mouse IgG









- Multiplexed format independently monitors the activities of 2 kinases
- Example shows preferential inhibition of ERK-2 by K252a, and c-SRC by Staurosporine

❿ Conclusions

- Activity of the entire MAPK cascade or any part of it can be monitored using the MSD Multi-Array platform.
- Whole proteins or short synthetic peptides can be employed as substrates.
- Assay protocols are suitable for HTS applications.
- Multiple kinases can be assayed simultaneously in a single sample by using specific target substrates immobilized on a MSD Multi-Spot plate.

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