# High-Throughput, Multi-Array™ Assays to Detect Binding of SH2 Domain Proteins to Phosphoproteins

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## Abstract

Interactions between adapter proteins and phosphoproteins are important in signaling cascades in both normal and pathological states. For example, the epidermal growth factor receptor (EGFR) contains several tyrosine residues that, when phosphorylated, serve as docking sites for SH2 domain proteins such as Grb2 and Shc. We have developed a novel assay that quantifies the activation level of EGFR through the binding of SH2 domain proteins to specific phosphorylated residues. The assay monitors EGFR phosphorylation events particular to a signal transduction pathway. A second assay has been developed for high throughput screening for inhibitors of adapter protein binding to activated receptors. The assays immobilize synthetic phosphopeptides corresponding to portions of a receptor on MSD Multi-Array™ or Multi-Spot™ plates. These phosphopeptides are challenged with SH2 domain protein(s) labeled with an electrochemiluminescent label. The assays can measure binding interactions that range from low picomolar to low micromolar.

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## ❷ Meso Scale Discovery Multi-Array Technology

SECTOR™ Imager 6000

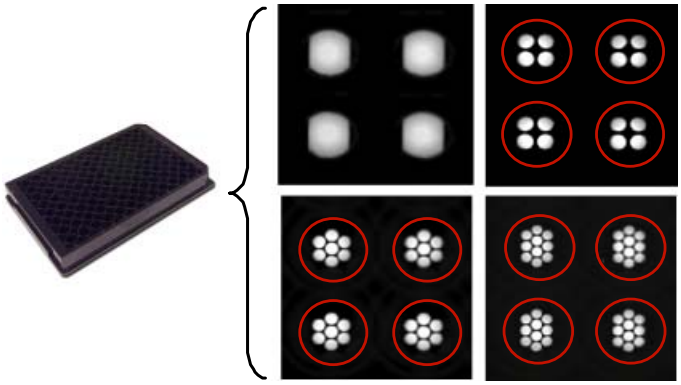
### Instrument Features

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

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.



MSD™

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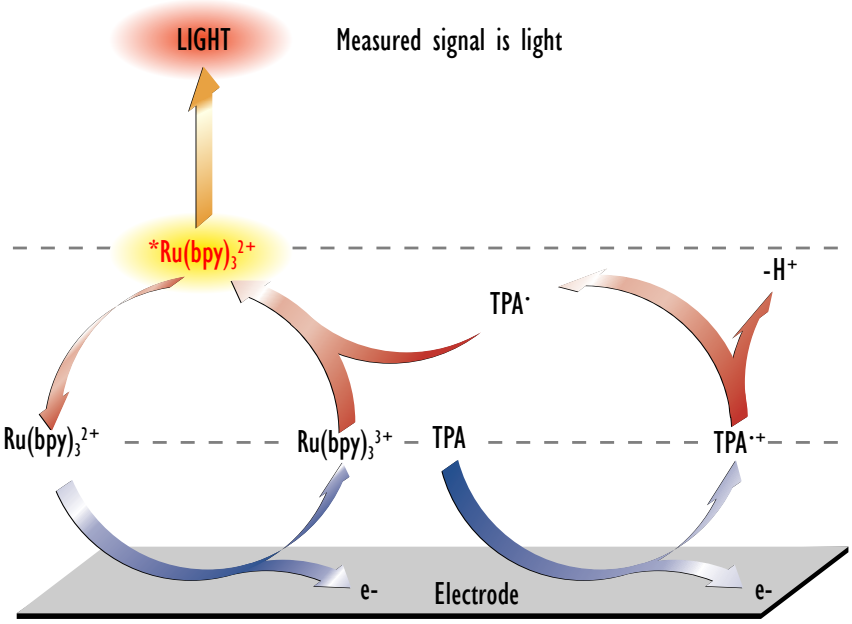
## ❸ Electrochemiluminescence (ECL)

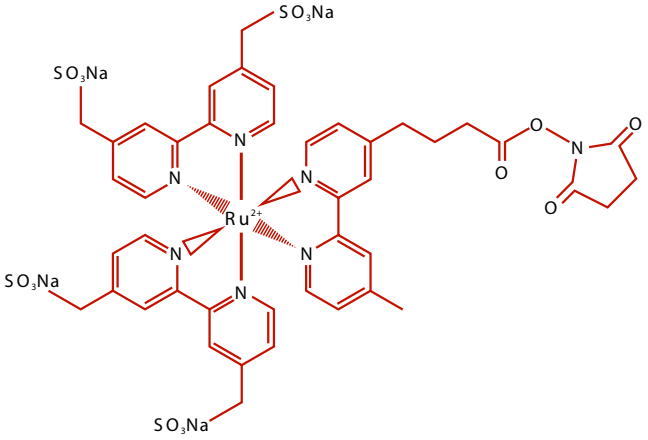



**Luminescence**
Emitting Light

**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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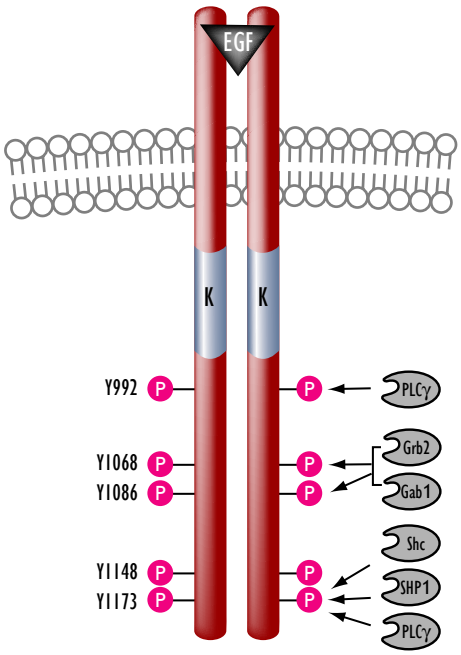
## ❹ Association of SH2 Domain Proteins to EGFR




| | |
|---|---|
| 992 | DADEYLIPQQ |
| 1068 | PVPEYINQSV |
| 1086 | QNPVYHNQPL |
| 1148 | DNPDYQQDFF |
| 1173 | ENAEYLRVAP |

Binding of adapter proteins to phosphorylated receptor tyrosine residues are critical to a number of important signaling pathways. Binding or "docking" is achieved by SH2 (src-homology 2) or PTB (phosphotyrsine binding) domains that recognize phosphotyrosine residues located within distinct amino acid contexts. The specificity of these interactions allows for the development of screening strategies for inhibitors of particular protein/protein interactions.

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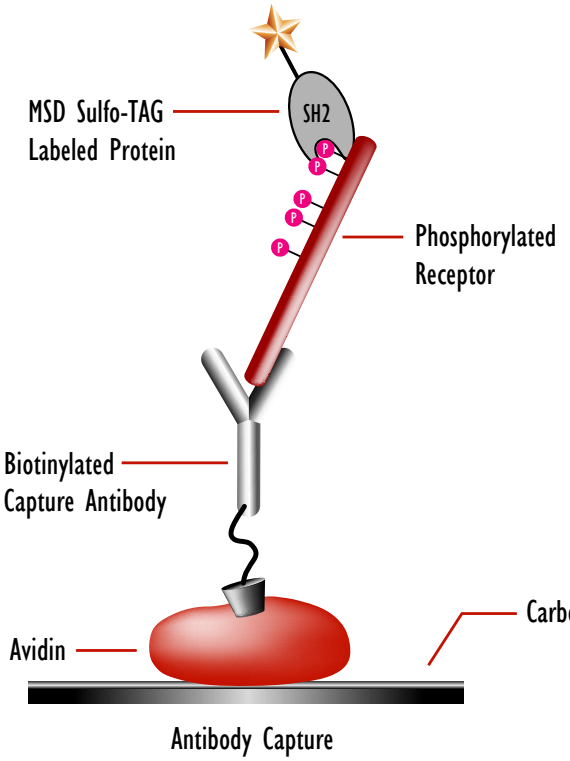
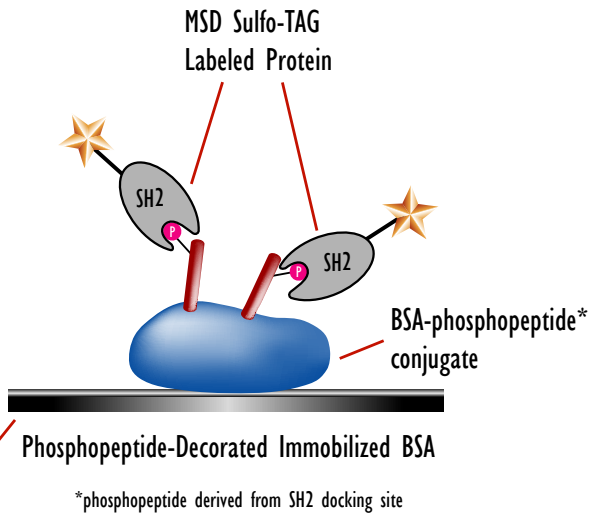
## ❺ Assay Formats

### Autophosphorylation Assay (EGFR)




### Adapter Protein Binding Assay




*phosphopeptide derived from SH2 docking site

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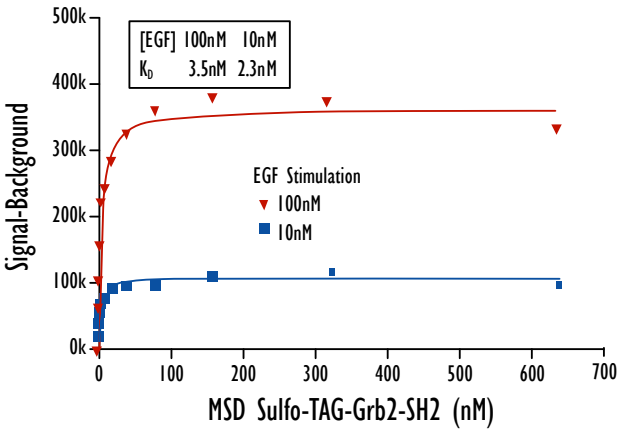
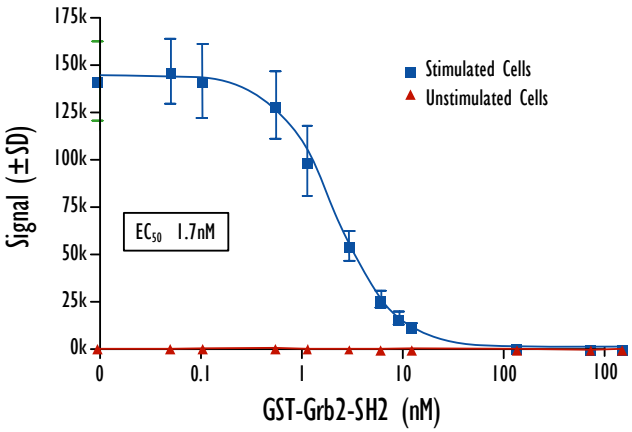
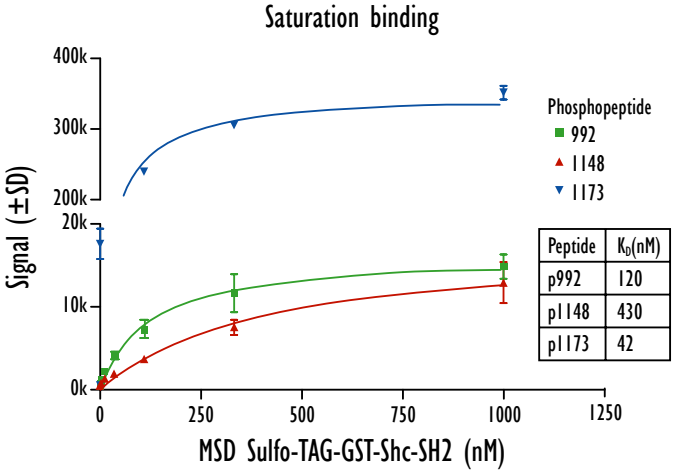
## 6 Detection of Activated EGFR by the Association of Grb2




EGF-treated cells were lysed *in situ* in multi-well plates. Portions of the lysate were transferred to avidin coated Multi-Array plates containing a biotinylated EGFR capture antibody. Left panel: MSD Sulfo-TAG-labeled recombinant GST-Grb2 SH2 domain protein was then added to the wells at the concentrations indicated. Right panel: Competition with unlabeled GST-Grb2 SH2 protein (MSD Sulfo-TAG-labeled species at 3nM).

## 7 Binding of the Shc SH2 Domain to EGFR Phosphopeptides




| Peptide | $K_D$(nM) |
|---|---|
| p992 | 120 |
| p1148 | 430 |
| p1173 | 42 |

BSA with coupled phosphopeptides was deposited onto the surface of a Multi-Array plate. MSD Sulfo-TAG-labeled recombinant GST-Shc SH2 domain protein was then added to the wells at the concentrations indicated. The plate was incubated at room temperature for 4 hours, washed and MSD Read Buffer added. It was then imaged on the SECTOR Imager 6000. The results are consistent with molecular genetic evidence that indicates the Shc SH2 domain preferentially binds to pY1173.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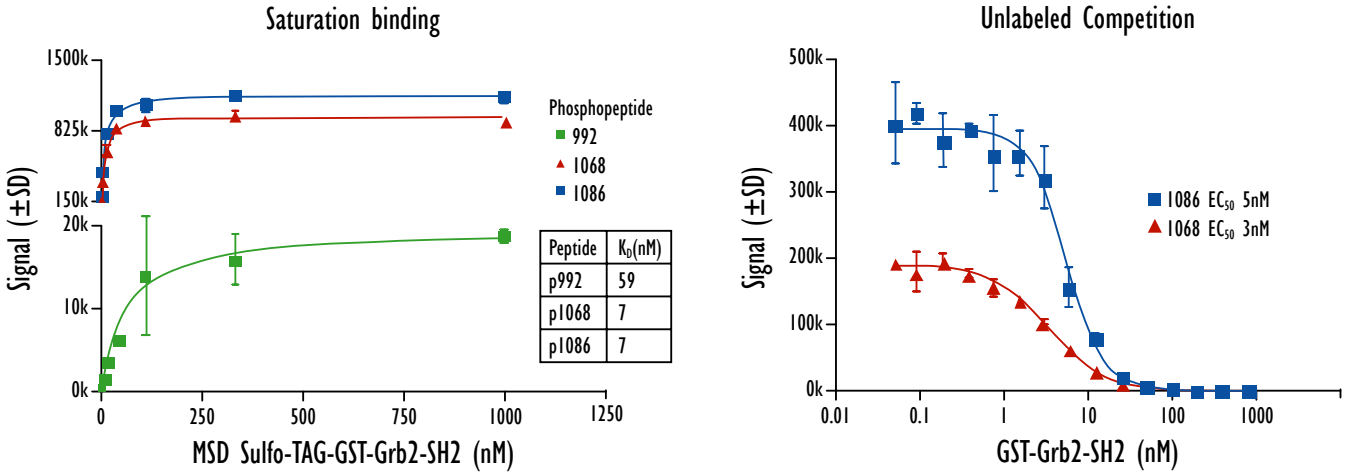
## 8 Binding of the Grb2 SH2 Domain to EGFR Phosphopeptides




BSA with coupled phosphopeptides was deposited onto the surface of a Multi-Array 384-well plate. Left panel: MSD Sulfo-TAG-labeled recombinant GST-Grb2 SH2 domain protein was then added to the wells at the concentrations indicated. The plate was incubated at room temperature for 4 hours, washed and MSD Read Buffer added. It was then imaged on the SECTOR Imager 6000. Right panel: Competition with unlabeled GST-Grb2 SH2 protein (MSD Sulfo-TAG-GST-Grb2-SH2 species at 6 nM). The results are consistent with those in the literature that have shown that Grb2 binds to both pY1068 and pY1086 using molecular genetic methods.

## 9 Conclusions

- A cell based assay has been developed that reports the phosphorylation status of EGFR through "docking" of specific adapter proteins.
- The use of adapter proteins allows one to monitor phosphorylation events associated with a particular signal transduction pathway.
- Isolated phosphopeptides can be used to quantify the binding affinity of SH2 domain proteins to specific receptor residues.
- Both assays have been developed so as to facilitate HTS efforts to identify novel small molecules that inhibit specific SH2/phosphopeptide interactions relevant to individual signaling pathways.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