A High-Throughput Replacement for Western Blots: Protein Expression Analysis Using the MSD Multi-Array™ Platform

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Abstract

We describe high-throughput protein expression assays that are quantitative, rapid and sensitive. These assays provide alternatives to existing methods such as Western blot analysis and immunofluorescence techniques, which are labor-intensive, low in throughput, and time-consuming. In one procedure, cell lysates are adsorbed directly in the wells of MSD Multi-Array™ microplates; another format uses specific capture antibodies to increase sensitivity. Both procedures detect specific proteins by using an antibody labeled with an electrochemiluminescence reporter. We present two examples that demonstrate the utility of these assays: the identification of HEK293 clones that over-express the human vascular endothelial growth factor receptor 2 (VEGFR2), and the detection of epidermal growth factor receptor (EGFR) in an established carcinoma cell line. The assay can also be used to quantify the abundance of particular post-translational states of proteins of interest.

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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

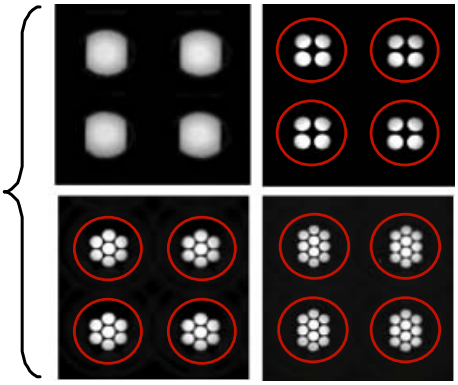

Plate Features

- 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.

MSD™

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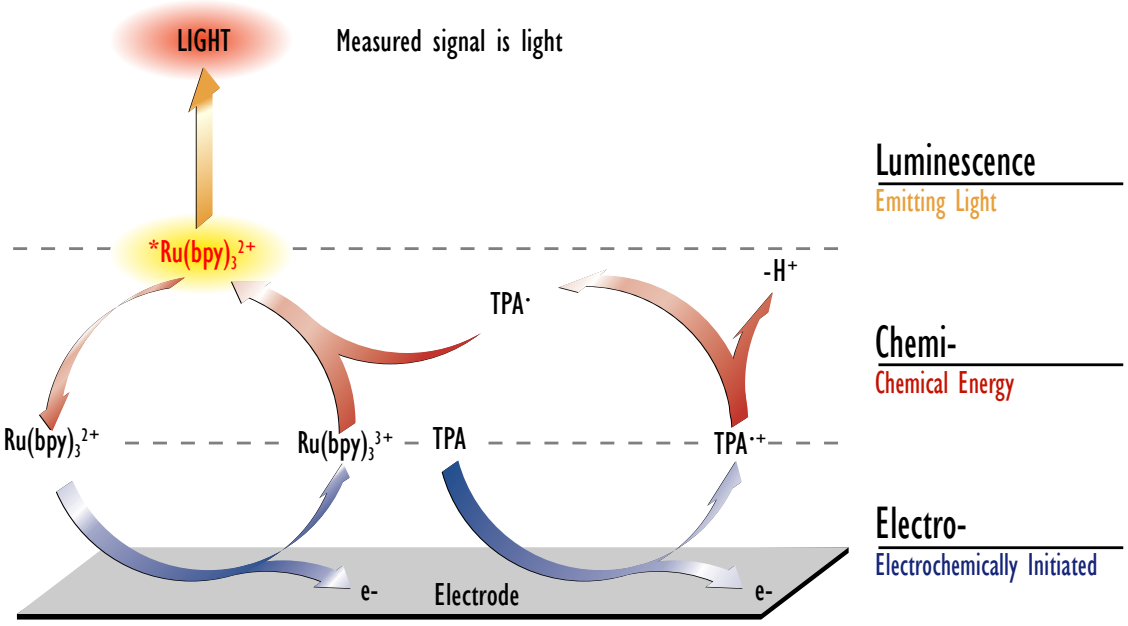
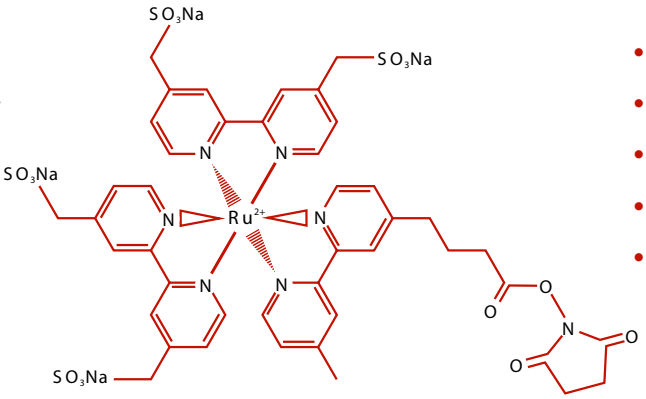
❸ Electrochemiluminescence (ECL)



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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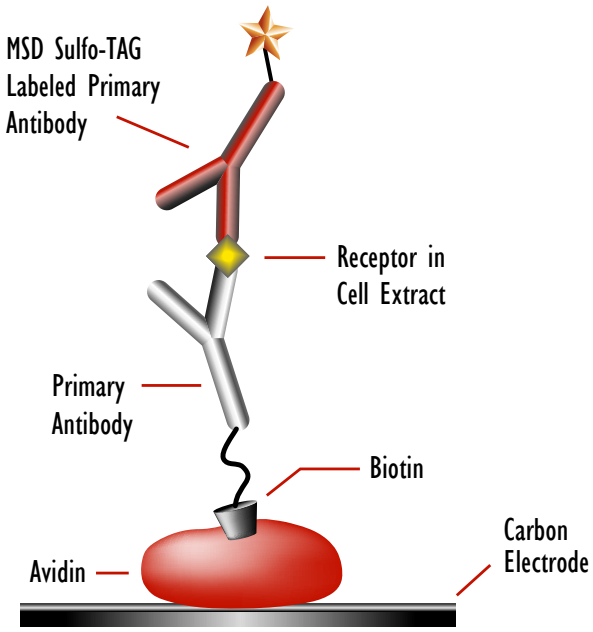
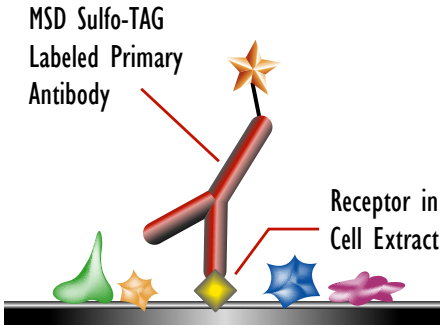
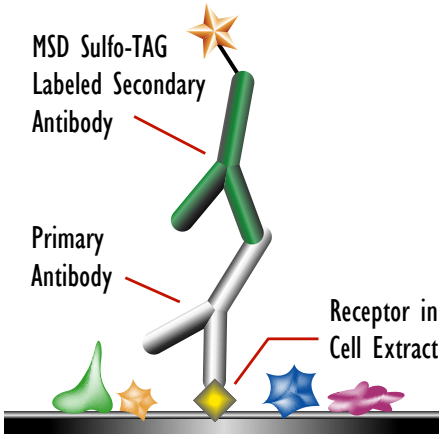
❹ Multiple Assay Formats



Two Antibody Method



One Antibody/Direct Detection



One Antibody/Indirect Detection

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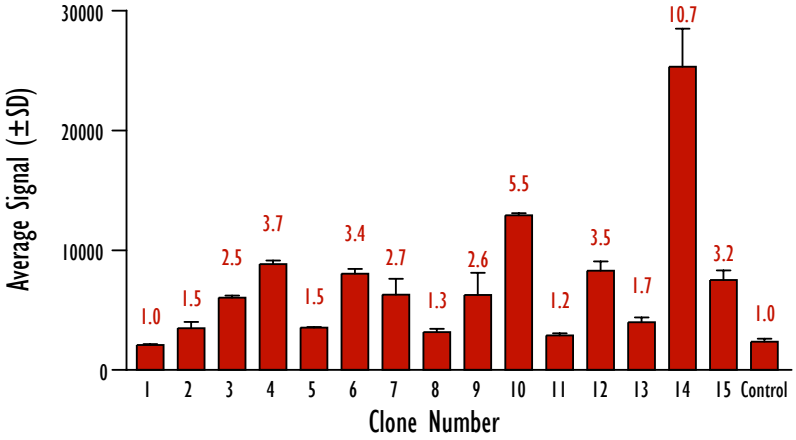
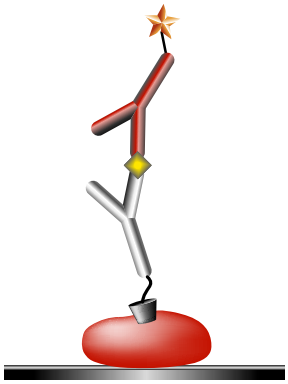
5 Screening VEGFR2-Expressing Clones with the Two Antibody Method





Detection of VEGFR2 in cell extracts from clonal isolates using the two antibody protocol. Whole cell extracts (10 µg protein, equivalent to 20,000 cells) were prepared from clonal isolates of HEK293 cells that had been transfected with a VEGFR2-expression plasmid. The cell extracts were added to the wells of SECTOR PR Multi-Array Avidin Coated High Bind plates after a biotinylated capture antibody had been immobilized on the surface. VEGFR2 expression in these clones correlated with VEGF-binding activity as determined using a receptor-ligand binding assay. Signal to background ratios are reported above each bar graph.

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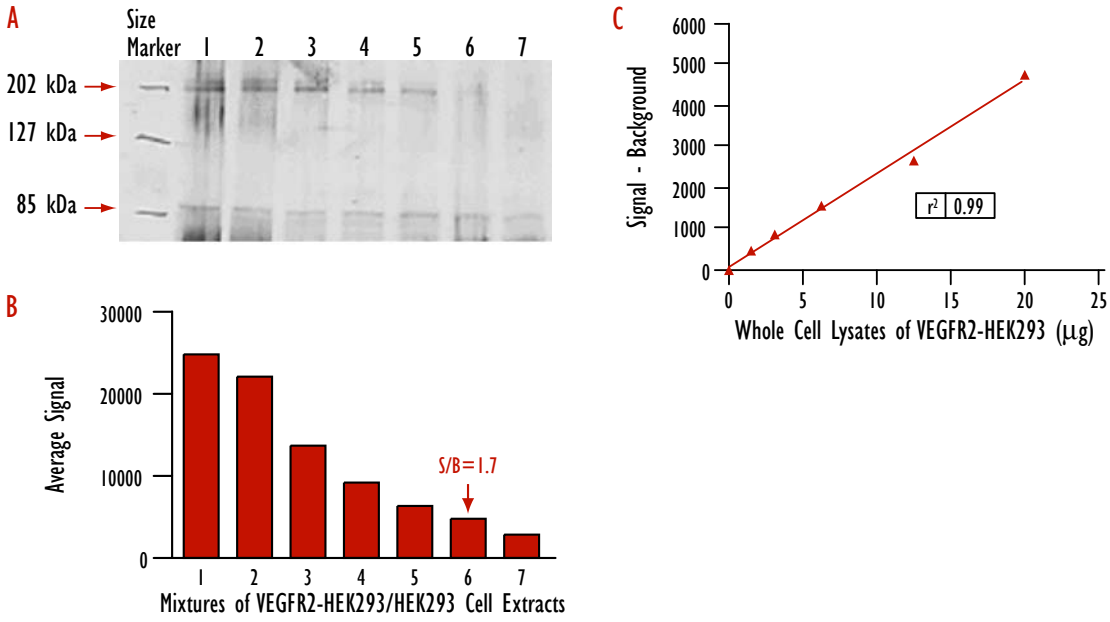
6 The Protein Expression Assay is Sensitive and Quantitative



Sensitivity of the protein expression assay as compared to Western blot analysis. Mixtures of cell extracts from a VEGFR2-expressing HEK293 clone (VEGFR2-HEK293) and a non-expressing control (HEK293) were prepared at the following ratios (ug total lysate protein): lane 1, 25:0; lane 2, 20:5; lane 3, 12.5:12.5; lane 4, 6.25:18.75; lane 5, 3.1:21.9; lane 6, 1.5:23.5; lane 7, 0:25. The mixtures (25 ug/sample) were analyzed by (A) Western blot analysis, or (B) the MSD protein expression assay. (C) The data from the Multi-Array protein expression assay show linear correlation of signal up to 20 µg extract protein. Signals are readily converted to numbers of receptors per cell, providing an estimate of 2 X 10^4 VEGFR2 molecules per cell in this cloned cell line.

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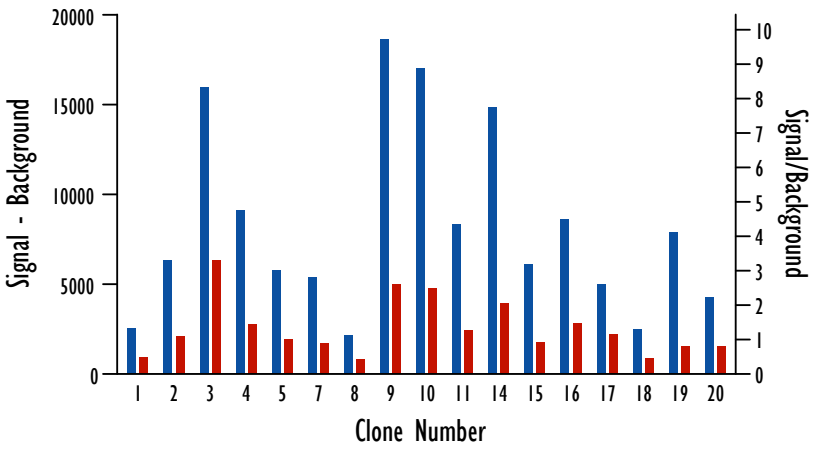
❼ Screening VEGFR2-Expressing Clones with the One Antibody Method



Detection of VEGFR2 in cell extracts from clonal isolates using the one antibody protocol for quantification of protein expression. Whole cell extracts (0.125 (red) or 0.5 (blue) µg protein, corresponding to 310 or 1250 cells, respectively) of HEK293 clonal isolates were analyzed using Multi-Array High Bind plates. The VEGFR2-expression differences measured using the one antibody approach were confirmed through VEGF-binding assays and correlate with the data obtained with the two antibody approach (Figure 5). The number of VEGFR2 molecules expressed per cell for the highest expressing clone, clone 9, was determined to be 3 X 10^6.

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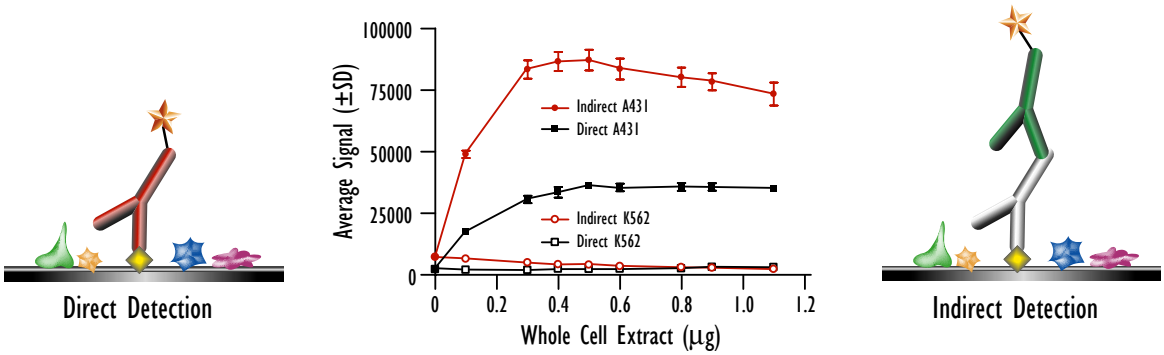
8 Detection of EGFR in A431 Cells



Application of the one-antibody method to detection of EGFR in A431 cell extracts. The direct and indirect reporter antibody methods are compared. Whole cells extracts of A431 (EGFR positive) and K562 (EGFR negative) cells were applied directly to the working electrodes of SECTOR PR Multi-Array High Bind plates. EGFR was detected using either 1 picomole of MSD Sulfo-TAG-labeled mouse-anti-human EGFR antibody (direct method), or 1 picomole unlabeled primary with 3 picomoles of MSD Sulfo-TAG-labeled anti-mouse IgG antibody (indirect method). Use of the indirect reporter protocol results in amplification of signal.

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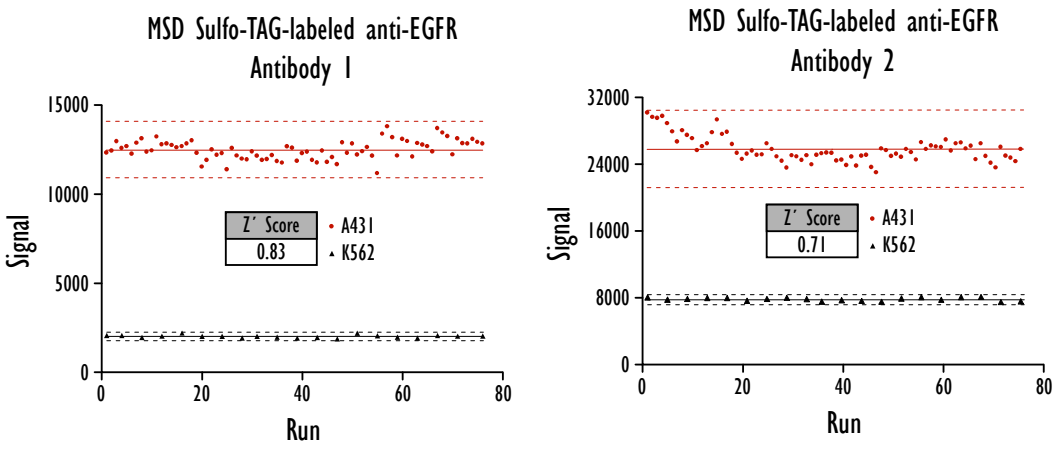
9 Assay Reproducibility and Validation — Z′ Score



Z′ score analysis with the one-antibody approach for EGFR detection (non-washed assay). The EGFR detection assay was carried out using 0.1 µg of A431 and K562 extracts on a Multi-Array High Bind plate. Two antibodies with differing label densities were tested. Acceptable Z′ scores were obtained in both cases despite the differences in overall signal output. The data demonstrate low variability and high signal to background ratios for this high throughput alternative to Western Blot analysis.

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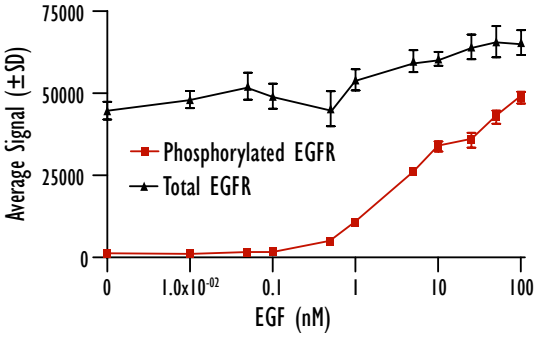
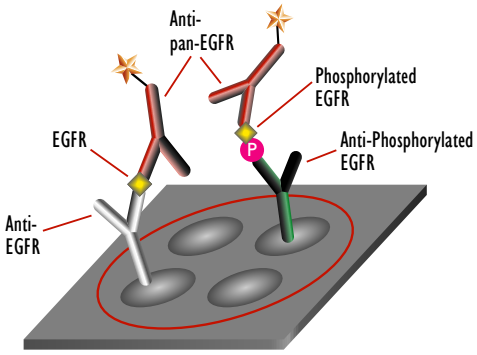
⑩ Detection of Post-translational Modification — Inducible Phosphorylation of EGFR





Detection of inducible EGFR autophosphorylation. Exposure of A431 cells to epidermal growth factor (EGF) results in inducible autophosphorylation of EGFR at specific tyrosine residues. The Multi-Array protein expression assay was modified to detect this phosphorylation event(s) using phosphotyrosine-specific antibodies to capture the receptor. Multi-Spot™ 4 spot plates facilitate simultaneous detection of total and phosphorylated EGFR from the same lysate through localization of distinct capture antibodies to individual electrodes in the array. Detection was afforded through the use of a MSD Sulfo-TAG-labeled anti-EGFR antibody that binds both populations of EGFR.

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11 Conclusions

- The Multi-Array protein expression assay is a high throughput, sensitive and efficient method for quantifying expression of a protein of interest.
- The assay is versatile and saves time and labor compared to other techniques.
- Small numbers of cell equivalents are sufficient, and usage of antibody reagents is found to be a fraction of that required for protocols such as Western blot analysis.
- The assay can easily be automated.
- Use of appropriate antibody reagents affords quantification of post-translational modifications such as inducible phosphorylation.
- The assay can be readily adapted to any proteins for which antibodies are available.