

Simple, Rapid Ligand-Binding Assay Using Immobilized Cellular Membranes

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Abstract

This poster presents a robust, receptor-ligand binding assay based upon a novel assay platform developed by Meso Scale Discovery™ (MSD™). MSD's platform combines array technologies and electrochemiluminescence detection to achieve ultra-fast, highly sensitive assays in a homogeneous format. Cellular membranes containing the EGF receptor were passively adsorbed to MSD proprietary coated electrodes embedded in multi-well plates. Binding of EGF to the EGF receptor was detected by inducing and measuring electrochemiluminescence from a labeled EGF ligand. Approximately 1000 cell equivalents per well yielded a signal to background ratio of 20. The observed K_D agrees with that reported in the literature and demonstrates that immobilization of the membranes and modification of the ligand do not alter the binding affinity. Binding specificity was confirmed with two inhibitors. The assay can be readily adapted to facilitate analysis of a broad array of receptor-ligand interactions.



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Multi-Array™ Technology

Unified technology platform with instruments, plates and reagents for drug discovery.

Combines the power of microarrays with the sensitivity of electrochemiluminescence.

96-, 384- and 1536 microplate formats.

Multi-Spot™ plates with high density arrays for multiplexing.

Sector HTS™ Instrument: High resolution imaging detection and robotic integration for HTS and large-scale proteomics.

Sector PR™ Instrument: Medium throughput benchtop reader for assay development, cellular and molecular biology, research in therapeutic areas, secondary screening, QC. Assays developed on Sector PR port to Sector HTS.



Sector HTS



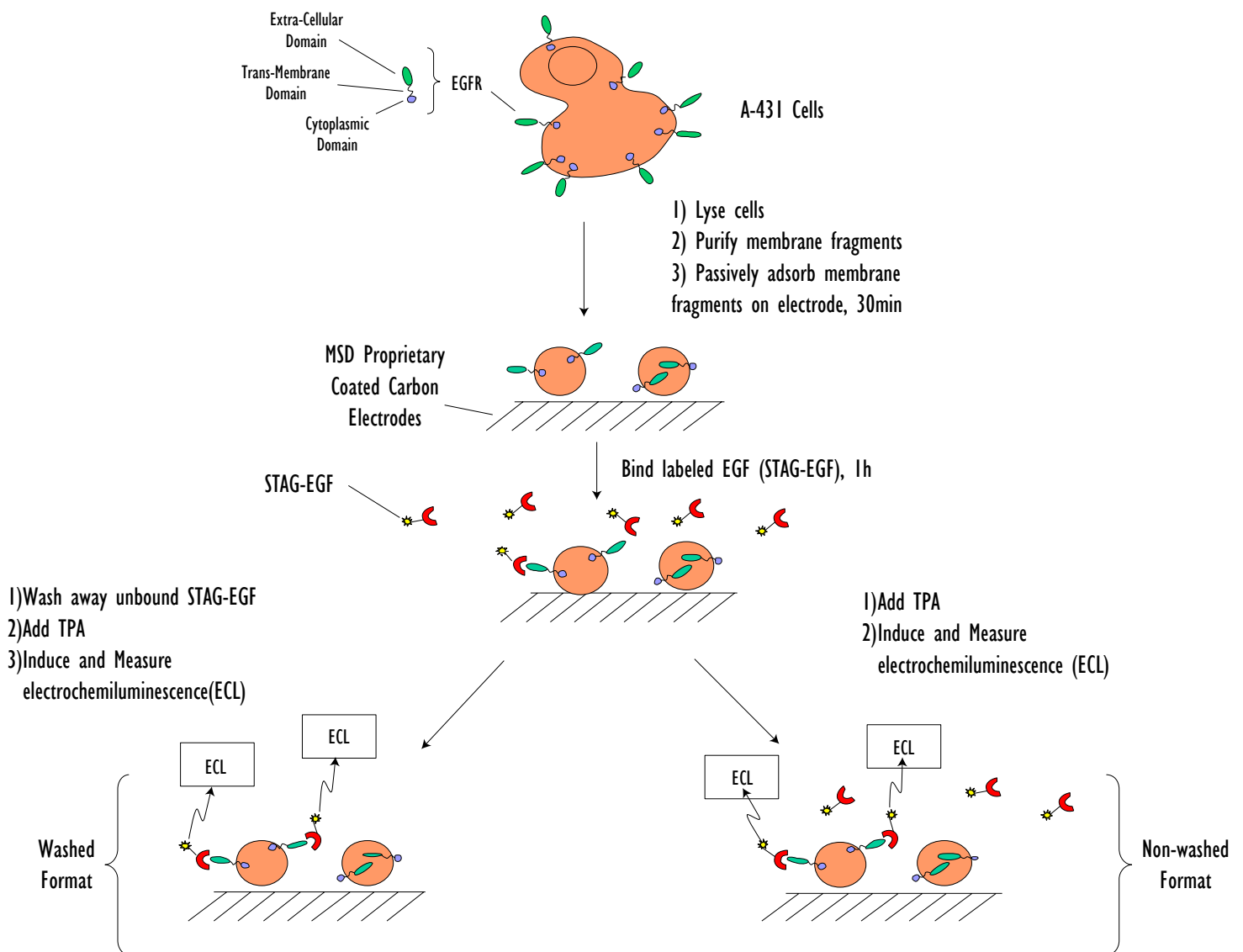
Sector PR



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EGF-EGFR Assay Format



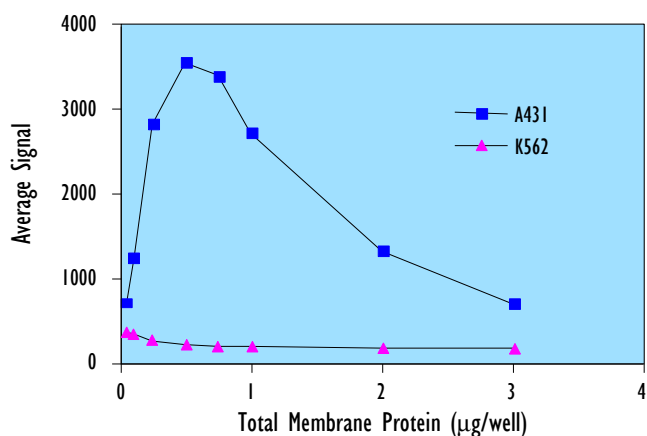
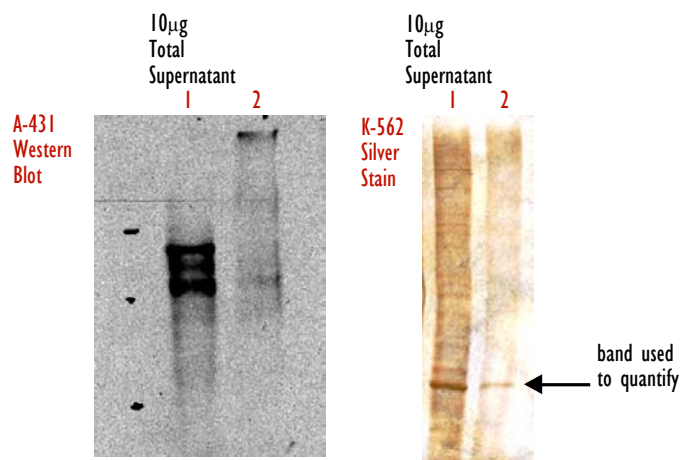
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Direct Immobilization of Biologically Active Membranes on Carbon Electrodes

Quantification of Membrane Immobilization on the Electrode Surface

Ten micrograms of total membrane protein was loaded directly onto the gel. For comparison, the supernatant containing the unbound fraction recovered after 10 μ g total membrane protein was incubated on the electrode for 30 min is shown. α -EGFR antibody was used for the western blot.



Optimal Mass Immobilized on the Electrode

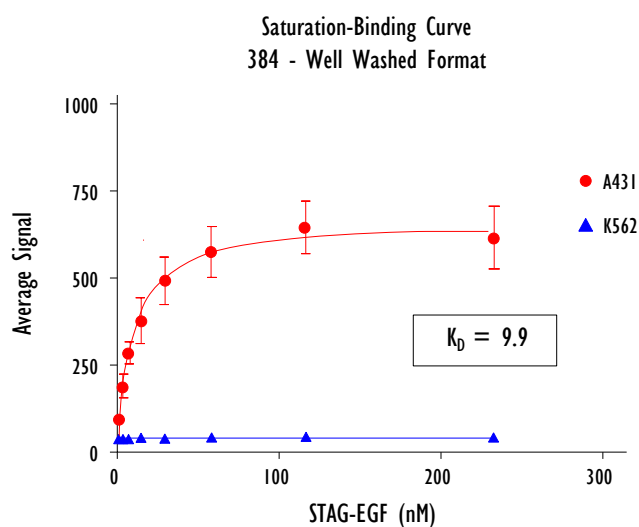
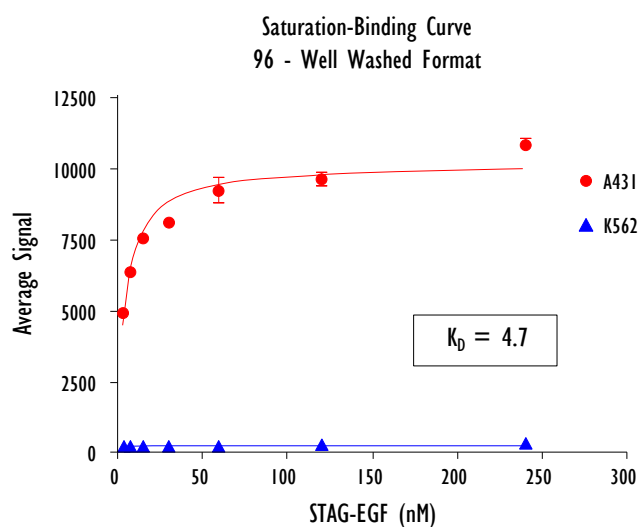
Titration of total membrane protein to determine optimal plating mass. In this 96-well washed format, membranes were immobilized onto MSD proprietary coated carbon electrodes in 5 μ L/well for 1 hour. Next, labeled ligand was added in 3% BSA for 1 hour. Unbound ligand was washed, followed by the addition of assay buffer just prior to electrochemiluminescence.



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Binding Characteristics of Membranes are Conserved After Immobilization on Carbon Electrodes



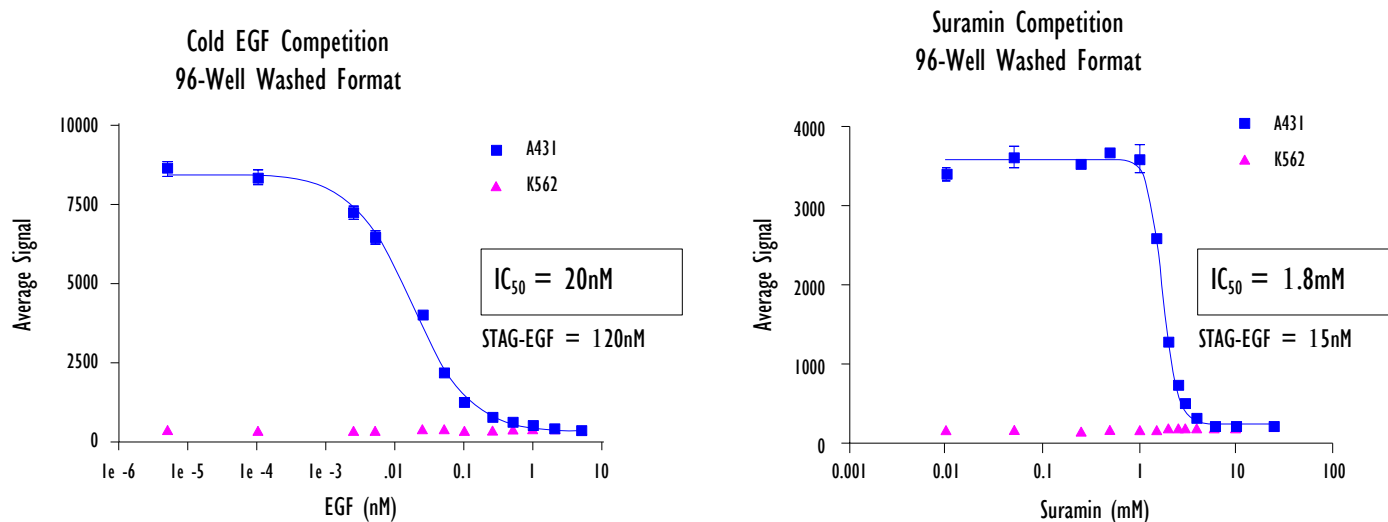
EGF was purchased (Sigma) and labeled with a sulfonated derivative of Ruthenium (II) tris-bipyridine (the electrochemiluminescent label, here abbreviated as STAG). STAG-EGF was purified by FPLC and the molecular weight of the labeled ligand was confirmed by mass-spectral analysis. Purified STAG-EGF was quantified by both a BCA protein assay and ruthenium absorbance at 455nm. As little as 1000 or 300 cell equivalents/well in the 96- and 384-well formats, respectively, produced maximum signal to background. The assay was performed using the standard protocol. The observed K_D values in both the 96- and 384-well assays were within 3-5 fold of that reported in scientific literature, demonstrating a very modest change in binding affinity of STAG-EGF relative to native EGF.



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Receptors in Immobilized Membranes Retain Sensitivity to Known Inhibitors



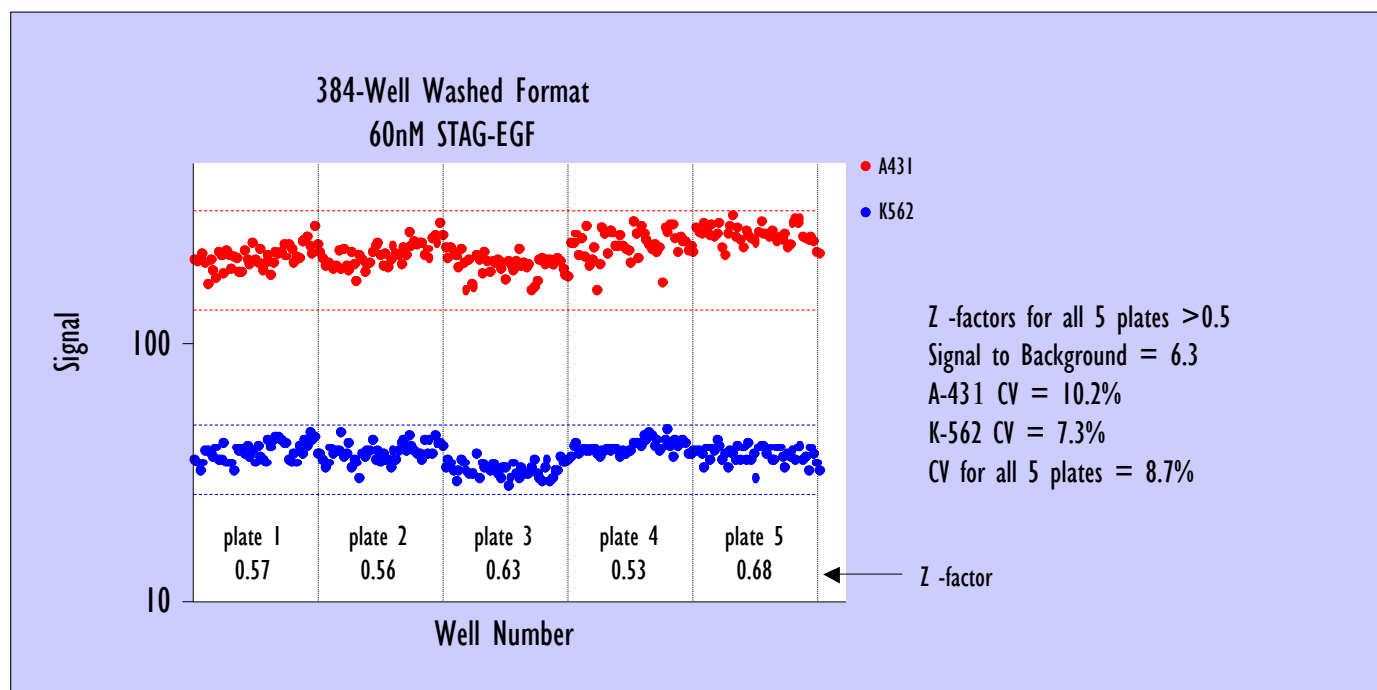
Receptor binding to STAG-EGF was assayed as a function of varying concentrations of unlabeled EGF. The observed IC_{50} value of the unmodified ligand relative to the STAG-EGF challenge is in agreement with the slightly greater K_D observed for the STAG-EGF. Suramin, another compound known to inhibit binding of several growth factor receptors, also inhibited STAG-EGF binding in the assay. The assay was conducted according to the standard protocol except that the labeled ligand and the inhibitor were incubated simultaneously in the plate.



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Membranes Immobilized on Carbon Electrodes are Compatible with HTS



In this 384-well, washed format, membranes were deposited with a PixSys™ instrument (Cartesian Technologies) and allowed to incubate on carbon electrodes for 30min. Labeled ligand, 60nM STAG-EGF, in 3% BSA was added by a RapidPlate instrument (Zymark) and incubated for 1 hour at ambient temperature. Unbound ligand was washed away, followed by the addition of assay buffer on the Rapid-Plate just prior to inducing electrochemiluminescence. The assay is adaptable to a fully integrated work system for high-throughput screens.



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Conclusion

1. We have developed a simple, rapid assay to detect the binding of the EGF ligand to EGFR via passive adsorption of biologically active membranes onto disposable, MSD proprietary coated carbon electrodes.
2. Quantitative membrane immobilization demonstrates biologically active A-431 and K-562 membranes. As little as $0.5\mu\text{g}$ (1000 cell equivalents) and $0.15\mu\text{g}$ (300 cell equivalents) total membrane protein/well, produced maximum signal to background in the 96- and 384-well assays, respectively.
3. Immobilization of the membranes onto MSD proprietary coated carbon electrodes and modification of the ligand result in a very modest alteration in binding affinity. Observed K_D s in both the washed and non-washed formats agree with that reported in literature, while characteristic competition curves with cold ligand and a known inhibitor demonstrate receptor-ligand binding specificity.
4. The assay is adaptable to completely automated 96- and 384-well formats for HTS. A 5-plate screen produced Z-factors > 0.5 /plate and interplate CV's = 8.7% in a 384-well washed format.

