

Antibody Screening Sandwich Assay by Label-free Epic[®] Technology

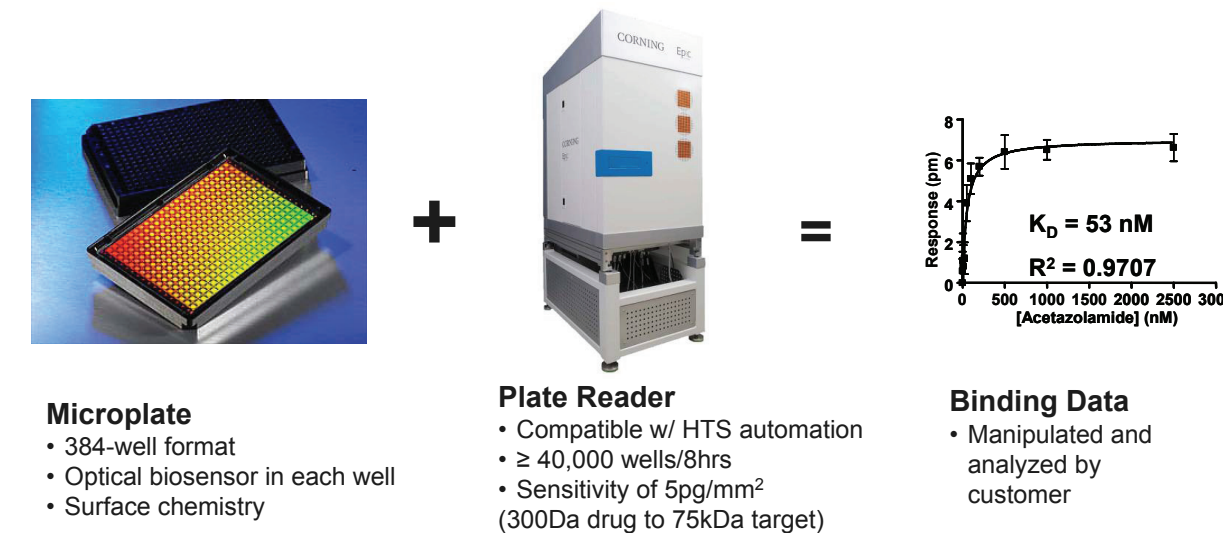
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Abstract

Antibody therapeutics represent the single fastest growing area of drug discovery and development. There are now more than 20 approved antibody therapeutics on the market with hundreds more in clinical trials and preclinical development. As a result of the increased interest in antibody therapeutics, there is currently a need for new tools to identify candidate antibodies as early in the drug discovery process as possible. These new tools must be i) sensitive enough to detect antibody-antigen interactions in complex biological samples; ii) flexible and easy to use and iii) high throughput for biopharmaceutical discovery needs. A label-free antibody competition assay was developed on the Corning[®] Epic[®] System to demonstrate the detection of the competitive binding to an immobilized receptor, and the ability to distinguish antibodies of distinct vs overlapping binding epitopes in complex samples. In these studies, the binding of three monoclonal antibodies against macrophage-colony stimulating factor receptor (M-CSF R) was investigated. The M-CSF receptor contained an Fc tag and was captured non-covalently via an anti-Fc antibody immobilized in the wells of a 384-well Epic[®] microplate. Binding of three different antibodies to the immobilized receptor was studied in the absence and presence of serum. When added individually, each antibody exhibited dose-dependent binding to the receptor. The assay exhibited a large signal window and a dynamic range from 0.1 μg/mL to >10 μg/mL in both assay buffer and culture medium containing 10% bovine serum. To test for competition, antibodies were added either sequentially or in batches to M-CSF R. Two of the antibodies were shown to compete for the same epitope on M-CSF R, consistent with the overlap in binding epitopes. The third antibody showed binding to a different epitope on the receptor and was non-competitive. The observed sensitivity of the assay suggests that the Epic[®] System can be used to screen hybridoma samples without the need for labeled secondary antibodies in a simple, high-throughput format.

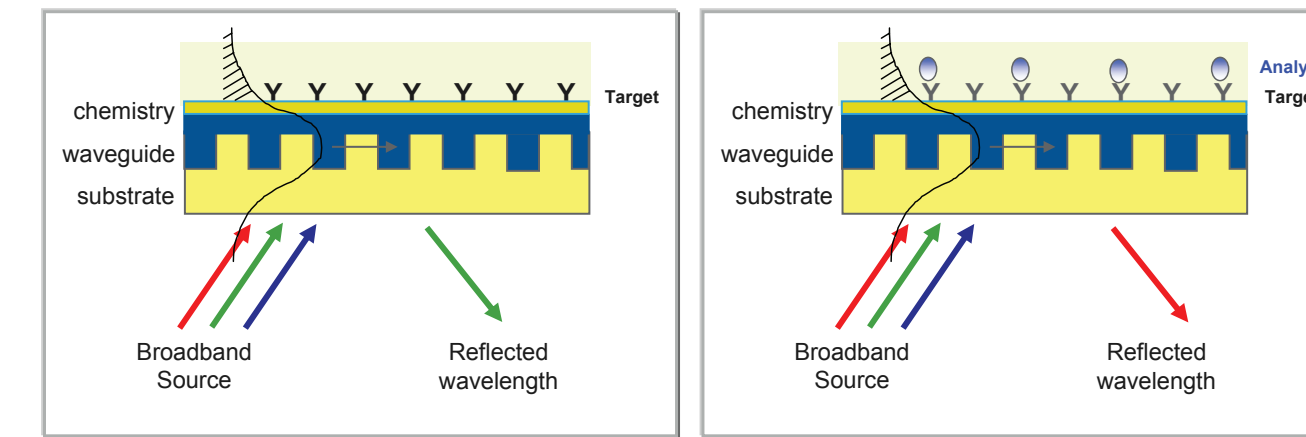
Corning[®] Epic[®] System Concept

The Corning Epic System is a high-throughput, label-free detection platform that consists of SBS-standard 384-well microplates with optical sensors inside each well, an HTS-compatible microplate reader and a set of label-independent assay protocols. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of "intractable" targets.



Operating Principle: Biochemical Assays

- Measures changes in index of refraction upon a binding event
- Change in index manifested by a shift in resonant wavelength



Label-free Antibody Assay Overview

Overnight or 1 hour antibody immobilization
 Wash/Block
 Baseline scan on the Epic[®] System
 Capture of M-CSF Receptor
 Optional off-line wash
 Scan on the Epic[®] System
 Binding of Antibody
 Optional off-line wash
 Scan on the Epic[®] System

Goat anti-human IgG-Fc polyclonal antibody is covalently immobilized to the surface of an Epic[®] microplate. Next, Fc-tagged M-CSF Receptor is non-covalently captured by the immobilized antibody. Two of the monoclonal antibodies compete for the same epitope on M-CSF Receptor, whereas the third antibody binds to an unique epitope. The specific binding of three monoclonal antibodies to the M-CSF Receptor is detected by Epic[®].

Results and Discussion: Optimization of M-CSF Receptor Capture

Response (μm) vs [M-CSF Receptor] (N=4)

- The non-covalent capture of M-CSF Receptor by GAH IgG-Fc was optimized using the following conditions:
 - M-CSF R concentration: 5, 10, 25 and 50 μg/mL
 - Nonspecific capture was monitored by using a negative control antibody (rabbit IgG).
 - The optimal capture conditions were identified as: 10 μg/mL M-CSF R

Optimization of Antibody Binding to Captured M-CSF Receptor

Response (μm) vs [Antibody #1] (ng/mL) (N=2)

- Sensitivity of antibody binding was improved in assay buffer containing surfactants.
- Assay dynamic range was 100 ng/ml to 10 μg/ml
- No significant reduction of specific antibody binding was observed following a post-binding wash (data not shown here)

Antibody Competition in PBS+CHAPS 1st Antibody Addition in a Batch Mode

Response (μm) vs Antibody (N=2)

- Each antibody binds to M-CSF Receptor when added individually (orange columns).
- Co-addition of Ab #1+2: No competition- both antibodies bind as indicated by 2X response.
- Co-addition of Ab #1+3: No competition- both antibodies bind as indicated by 2X response.
- Co-addition of Ab #2+3: Competition observed (1X response)

Antibody Competition in PBS+CHAPS 2nd Antibody Addition in a Serial Mode

Response (μm) vs Second Antibody (N=2)

- After the 1st antibody addition, a wash step was performed followed by the sequential addition of a second antibody.
- The expected results are observed:
 - Antibodies #2 and #3 show competition.
 - Antibody #1 does not compete with Ab#2 or Ab#3.

Antibody Dose-Dependent Binding in DMEM+10% FBS

Response (μm) vs [Antibody] (ng/mL) (N=2)

- Each antibody was added to non-covalently captured M-CSF Receptor in DMEM+10% FBS.
- Following antibody binding, the wells were washed 5X with PBS+0.02% CHAPS
- Dose-dependent binding for each antibody was observed in DMEM+10% FBS
- Assay dynamic range was 100 ng/ml to 10 μg/ml under the present conditions

Comparison of Antibody Dose-response Determined in Buffer and in DMEM+10% FBS

Antibody #1 Dose-response in PBS+CHAPS vs [Antibody #1] (ng/mL) (N=2)

Antibody #1 Dose-response in DMEM+10% FBS vs [Antibody #1] (ng/mL) (N=2)

- Antibody #1 was added to captured M-CSF Receptor in either assay buffer or DMEM+10% FBS
- Similar assay sensitivity was observed in assay buffer alone and in culture media+10% FBS

Antibody Competition in DMEM+10% FBS 1st Antibody Addition in a Batch Mode

Response (μm) vs Antibody (N=2)

- Each antibody binds to M-CSF Receptor when added individually (orange columns).
- Pre-incubation of Ab #1+2: No competition- both antibodies bind as indicated by 2X response.
- Pre-incubation of Ab #1+3: No competition- both antibodies bind as indicated by 2X response.
- Pre-incubation of Ab #2+3: Competition observed (1X response)

Antibody Competition in DMEM+10% FBS 2nd Antibody Addition in a Serial Mode

Response (μm) vs Antibody (N=2)

- Antibodies #2 and #3 always show competition, in agreement with sharing the same epitope
- Antibody #1 does not compete with Ab#2 or Ab#3, consistent with the antibodies binding to different epitopes in the competition assay

Summary

- The present data successfully demonstrate the principle of an antibody sandwich assay on the Epic[®] System
- Similar assay performance was observed in both assay buffer and in culture media plus 10% FBS
- Results demonstrate the successful immobilization of the functional GAH IgG-Fc antibody and the subsequent non-covalent capture of the M-CSF receptor
- Detection of mutually independent binding of antibodies to M-CSF receptor with/without 10% FBS was consistent with the different binding epitopes among the antibodies
- Detection of mutually exclusive binding to M-CSF receptor with/without 10% FBS was in agreement with the antibodies sharing the same epitope
- Binding of antibody in either assay buffer or DMEM+10% FBS can be detected successfully from 0.1 to 10 μg/ml
- The simple label-free assay format and capability of detecting binding in serum containing samples may enable its applications for antibody screening in complex biological samples