

# CORNING

Epic<sup>®</sup>  
system

## Development of an Antibody-Sandwich Assay for Hybridoma Screening Using the Corning Epic<sup>®</sup> System

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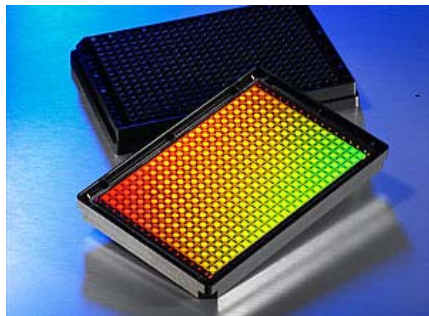
# Abstract

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 sensitive enough to detect antibody-antigen interactions in complex biological samples and they must be high throughput in order to screen the large number of hybridoma samples being generated for antibody discovery.

A label-free antibody sandwich assay was developed on the Corning® Epic® System to characterize competing and non-competing antibodies in a complex biological sample. 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 either assay buffer or culture medium containing 10% bovine serum. To test for competition, antibodies were added either sequentially or in batches to M-CSF R. The expected competition was observed when antibodies were added to the receptor either sequentially or in batch mode in the presence and absence of serum. Two of the antibodies were shown to compete for the same epitope on M-CSF R. The third antibody showed binding to a different epitope on the receptor and was non-competitive. Functional binding experiments were also performed with M-CSF (CSF-1), the natural ligand for M-CSF receptor. The ability of monoclonal antibodies to inhibit CSF-1 binding to M-CSF receptor was investigated. The observed sensitivity of the assay suggests that the Epic® System can be used to screen hybridoma samples for protein drug discovery in a high throughput manner with the relatively simple assay format offered by the label-free Epic® technology.

# Corning® Epic® System

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. The Epic System is applicable to both biochemical and cell-based assays, and enables high-throughput screening of “intractable” targets.



## Microplate

- 384-well format
- Optical biosensor in each well
- Surface chemistry

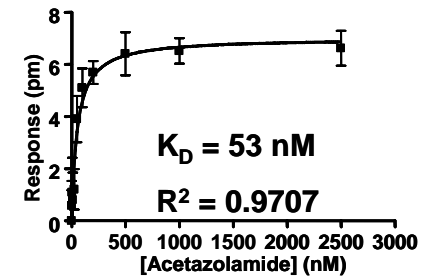
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## Plate Reader

- Compatible w/ HTS automation
- $\geq 40,000$  wells/8hrs
- Sensitivity of  $5\text{pg}/\text{mm}^2$   
(300Da drug to 75kDa target)

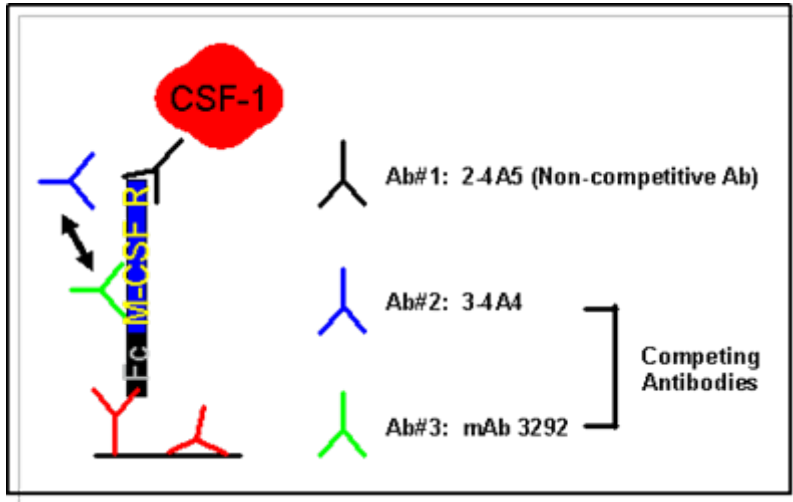
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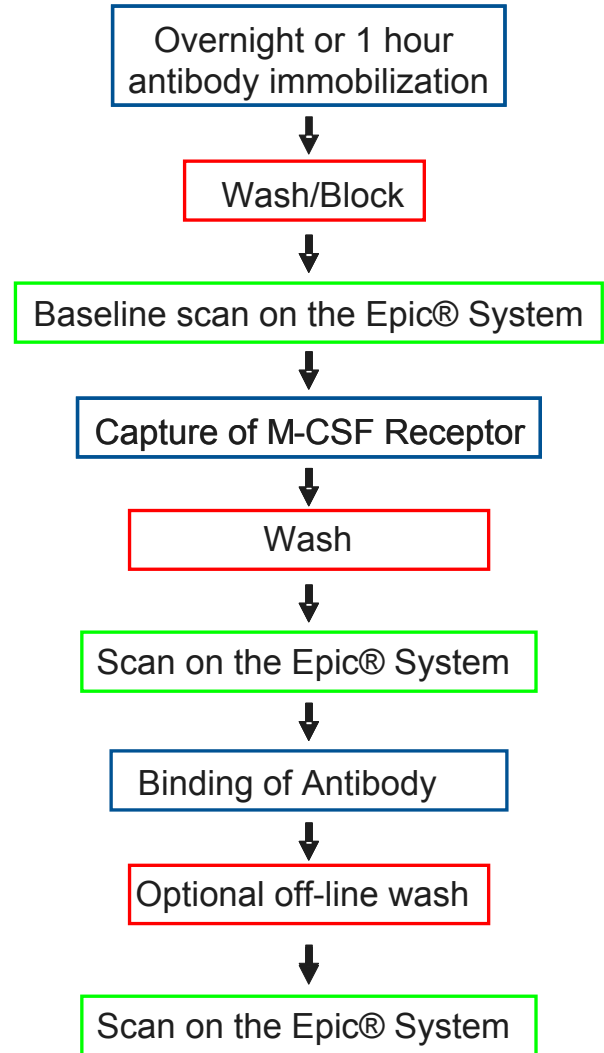
## Binding Data

- Manipulated and analyzed by customer

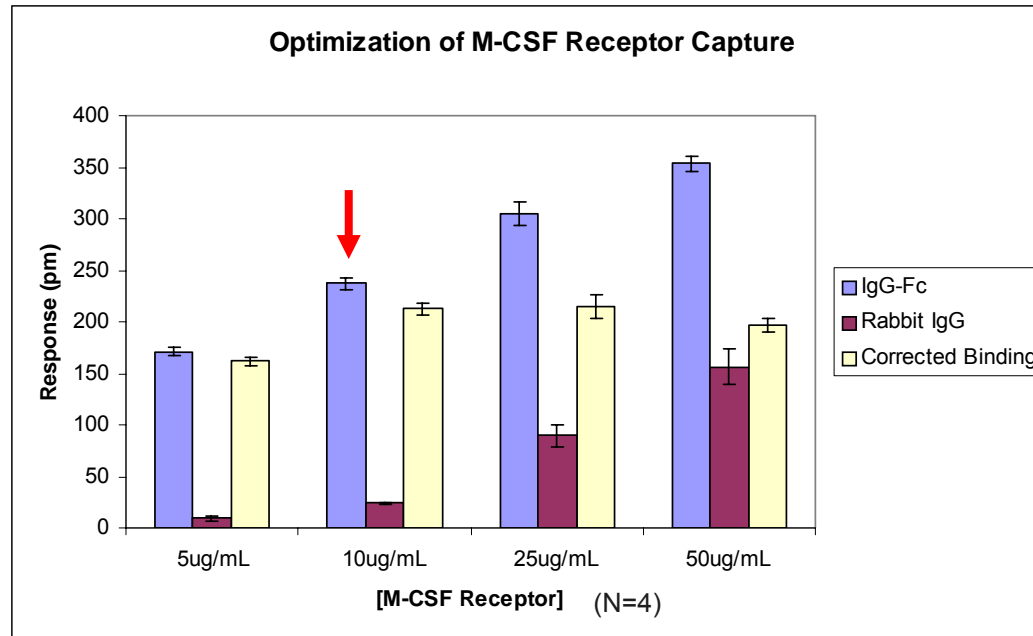
# Label-Free Antibody Assay Overview



Goat anti-human IgG-F<sub>c</sub> polyclonal antibody was covalently immobilized to the surface of an Epic® microplate. Next, F<sub>c</sub>-tagged M-CSF Receptor was 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 a unique epitope. The binding of three monoclonal antibodies to the M-CSF Receptor was detected by Epic®.

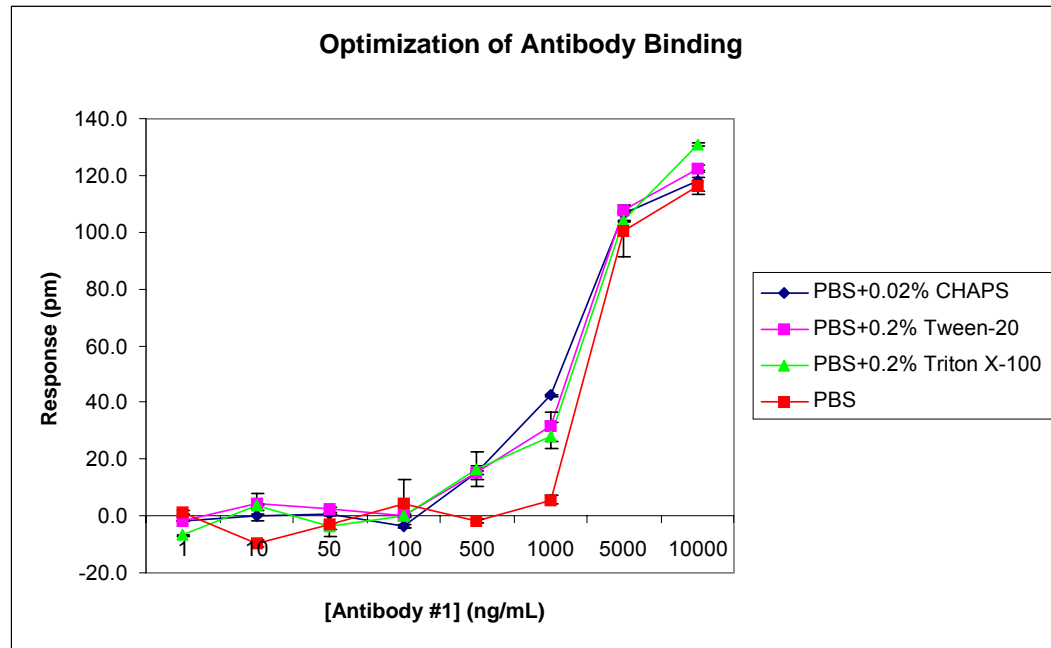


# Results and Discussion: Optimization of M-CSF Receptor Capture



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

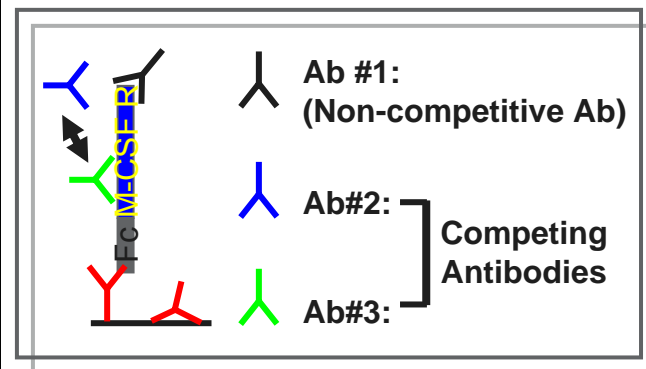
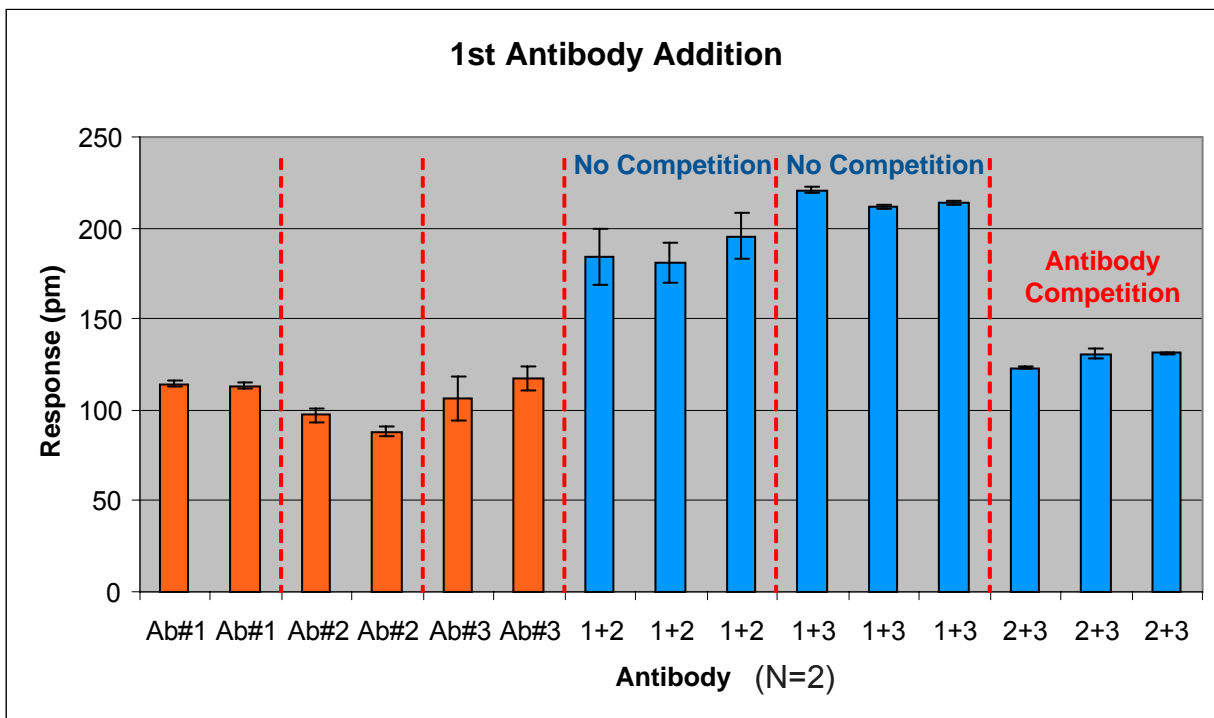
# Optimization of Antibody Binding to Captured M-CSF Receptor



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

# Antibody Competition in PBS+CHAPS

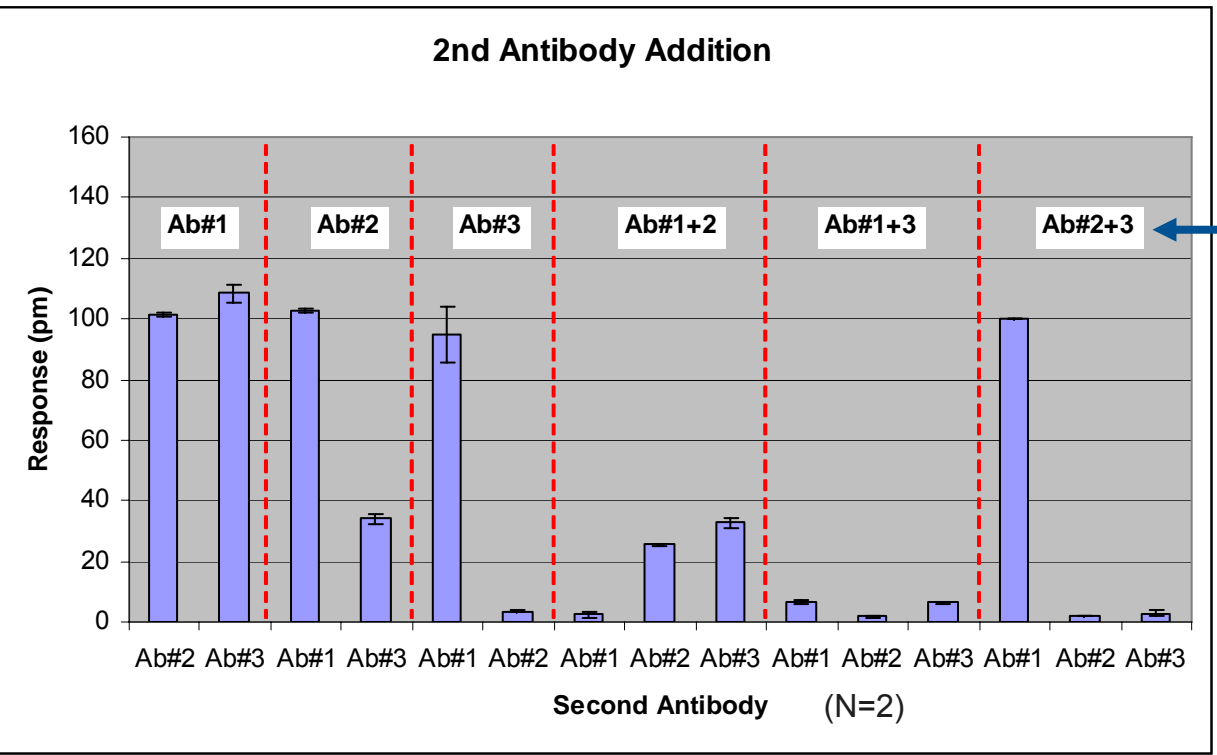
## 1<sup>st</sup> Antibody Addition in a Batch Mode



- 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

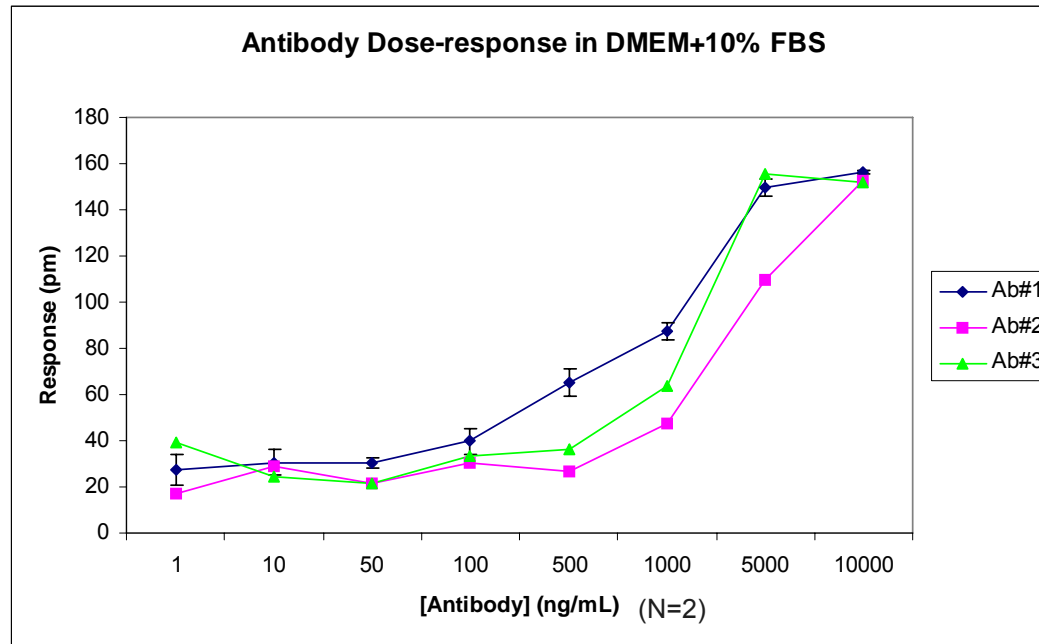
## 2<sup>nd</sup> Antibody Addition in a Serial Mode



Antibody added during first addition

- After the 1<sup>st</sup> 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.

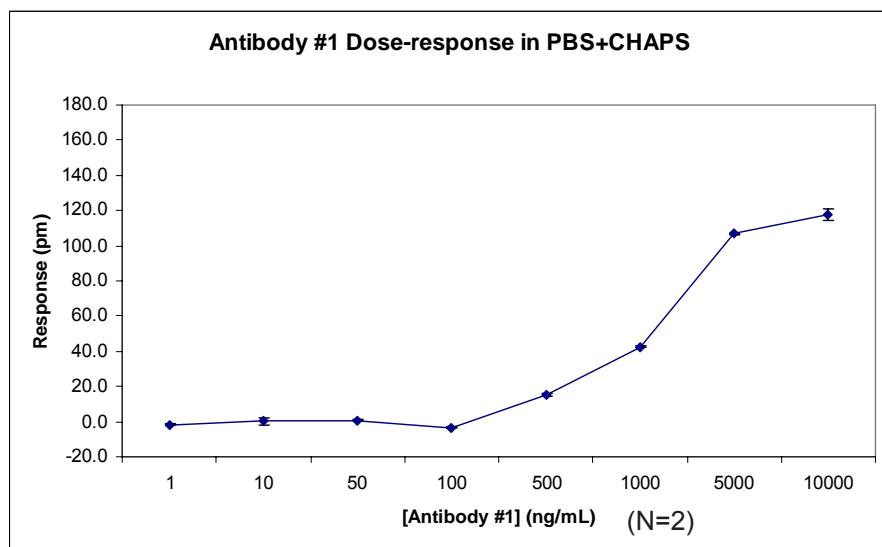
# Antibody Dose-Dependent Binding in DMEM+10% FBS



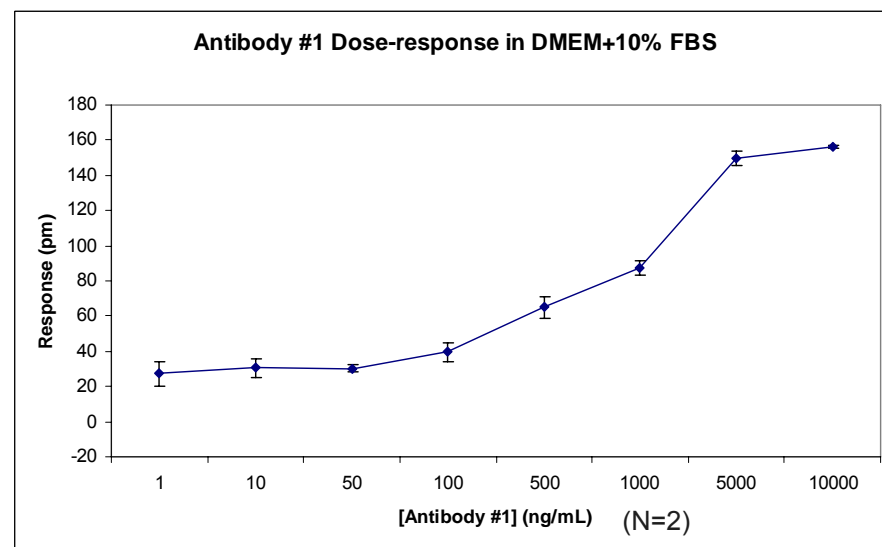
- Each antibody was added to non-covalently captured M-CSF Receptor in DMEM+10% FBS.
- Following antibody binding, the wells were washed 5X with PBS+0.02% CHAPS
- Dose-dependent binding for each antibody was observed in DMEM+10% FBS
- Assay dynamic range was 100 ng/mL to 10  $\mu$ g/mL under the present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

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

## Assay Buffer



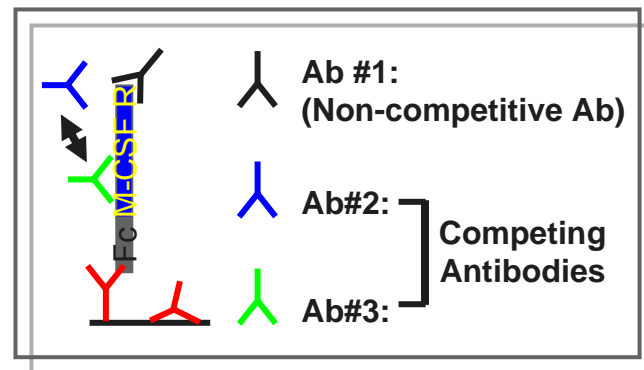
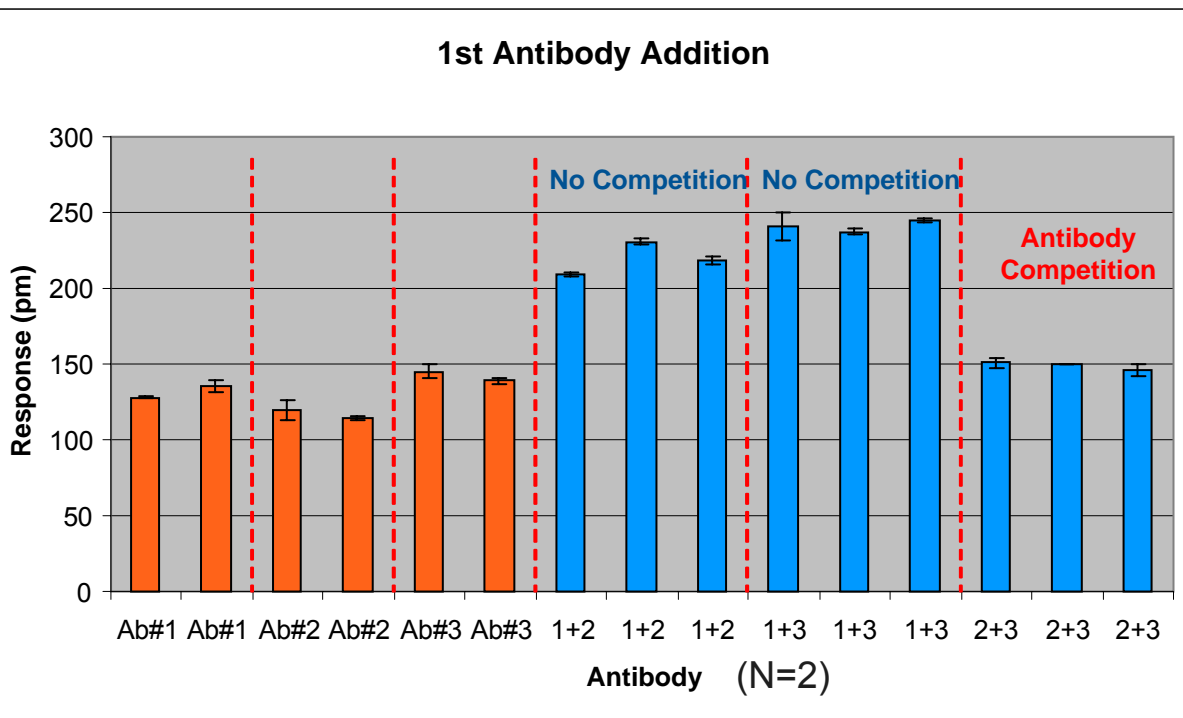
## DMEM+10% FBS



- 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

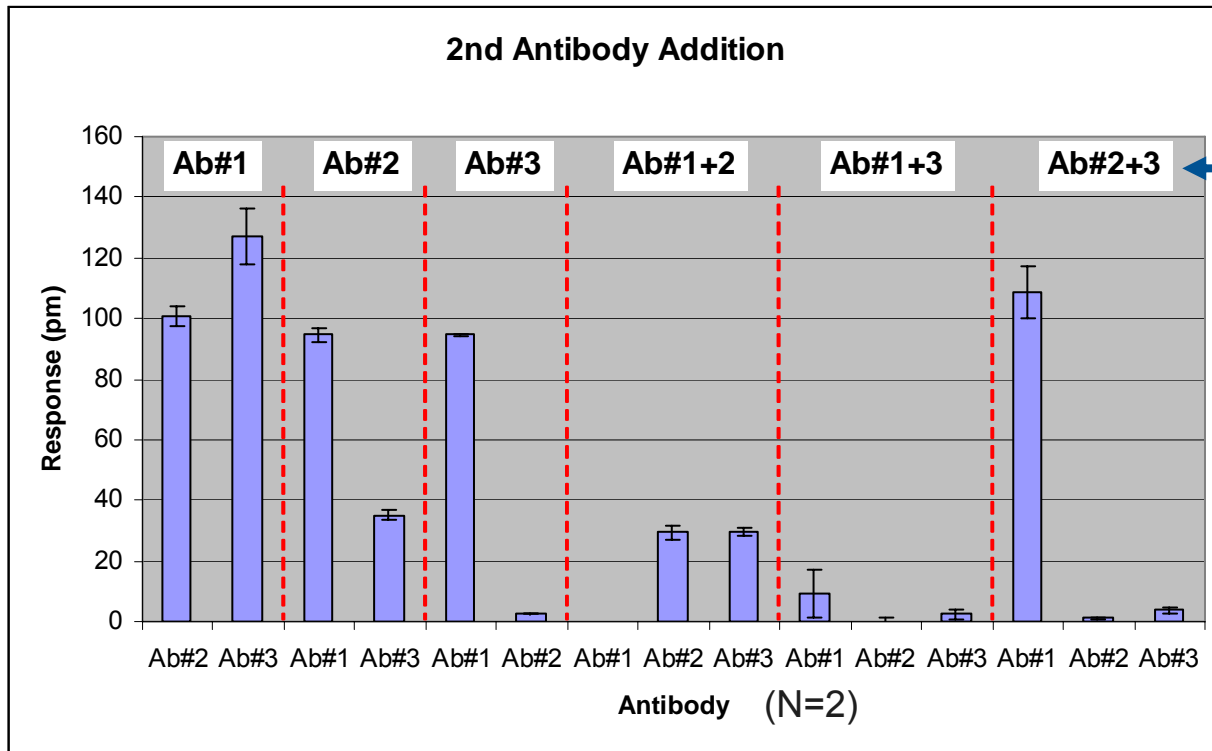
## 1<sup>st</sup> Antibody Addition in a Batch Mode



- 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

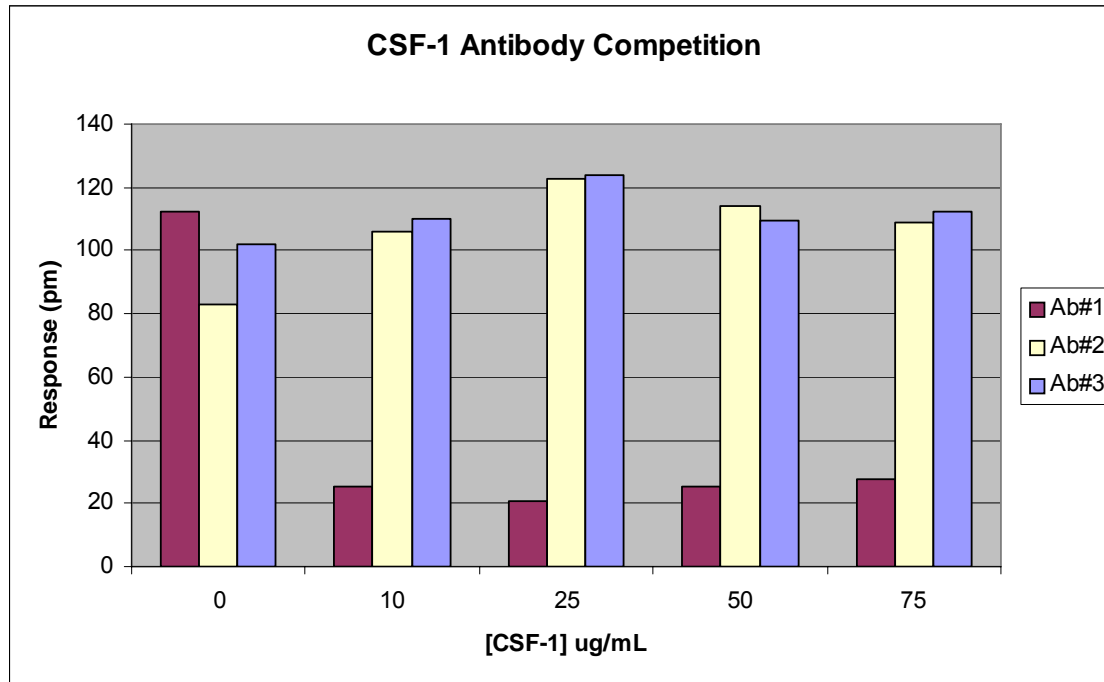
## 2<sup>nd</sup> Antibody Addition in a Serial Mode



Antibody added during first addition

- 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

# Functional Competition in the presence of CSF-1



- The ability of CSF-1 to block monoclonal antibody binding was tested.
- CSF-1 was added to captured M-CSF Receptor at the indicated concentrations.
- Each monoclonal Ab (5 $\mu$ g/mL) was then added to the M-CSF R/CSF-1 complex.
  
- CSF-1 blocks the ability of Antibody #1 to bind to M-CSF receptor.
- CSF-1 does not block Antibody #2 and #3 binding to M-CSF receptor.

# 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-F<sub>c</sub> 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
- A functional competition assay with CSF-1 yielded the expected results. Antibody #1 was unable to bind to M-CSF receptor in the presence of CSF-1. CSF-1 had no impact on the binding of Antibody #2 or Antibody #3 to M-CSF receptor.
- 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