



Generation of High Affinity Recombinant Anti-IL-6 Antibodies for Application in Bio-Plex® Assays

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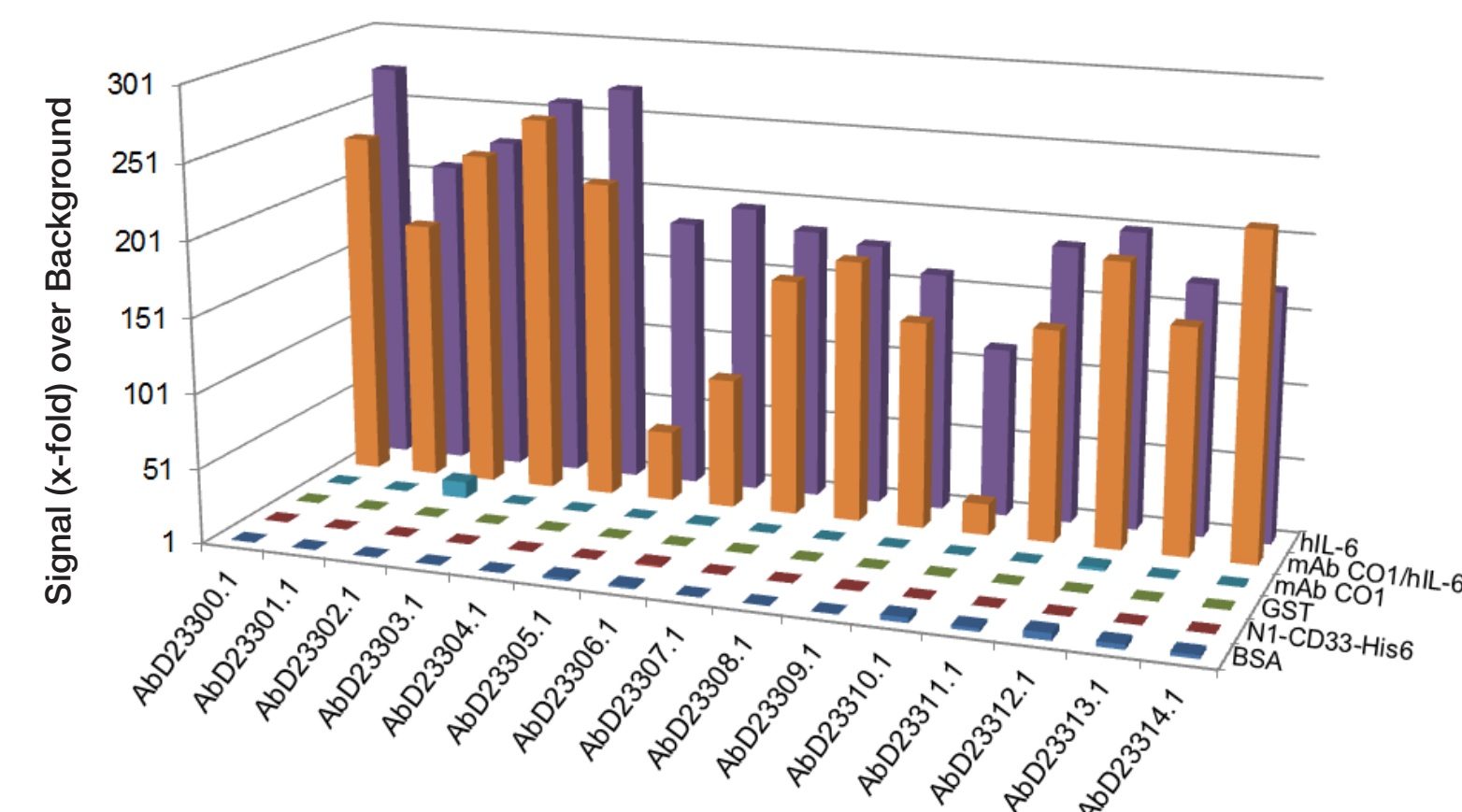
Introduction

Antibodies for research and diagnostic applications are still dominated by products derived from immunization of animals. The HuCAL® antibody technology, originally built for antibody drug development, has been offered by Bio-Rad since 2003 to develop custom recombinant monoclonal antibodies on demand. With the current library, HuCAL PLATINUM®, more than 45 billion specificities are used to select and purify antibodies against virtually any antigen in 8 weeks. Intelligent screening protocols are used to direct selections towards antibodies with specificities rarely reachable with traditional methods. The entire process is animal free; hence there are no restrictions on the antigens used. With recombinant antibody generation there is also a plethora of engineering possibilities including a choice of tags, labels and fusions to functional domains as well as conversion into different full immunoglobulin formats.

We have used guided selection to develop high affinity anti-human interleukin-6 (IL-6) antibodies, which form a sandwich pair with an existing Bio-Rad mouse monoclonal antibody. The IL-6 level in blood is very low (~1 pg/ml). Nevertheless, HuCAL antibodies with sufficient sensitivity could be selected from a standard antibody generation project. The best antibody pair, consisting of mouse monoclonal antibody CO1 as capture and HuCAL antibody AbD23302 with 20 pM affinity to IL-6 as detection antibody, is more sensitive than the current antibody pair used in several Bio-Rad Bio-Plex panels.

If even higher sensitivities are needed, the modular design of the HuCAL library allows rapid affinity maturation leading to antibodies with affinities in the single-digit picomolar or even sub-picomolar range.

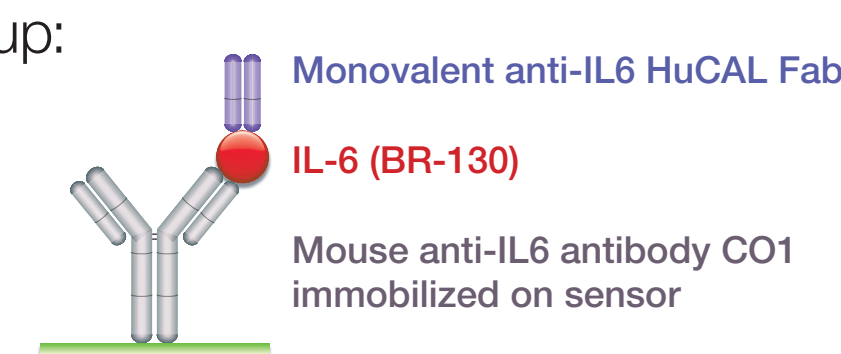
QC ELISA Results of Anti-hIL-6 Antibodies in Fab-FH Format



- AbD23300 – 23310 are from panning on hIL-6
- Most antibodies also work as detection antibodies on CO1/hIL-6 complex
- AbD23311 – 23314 are from panning on CO1/hIL-6 complex

Affinities of Best Antibodies

- Binding kinetics were measured on ProteOn XPR36 instrument
- Assay set-up:

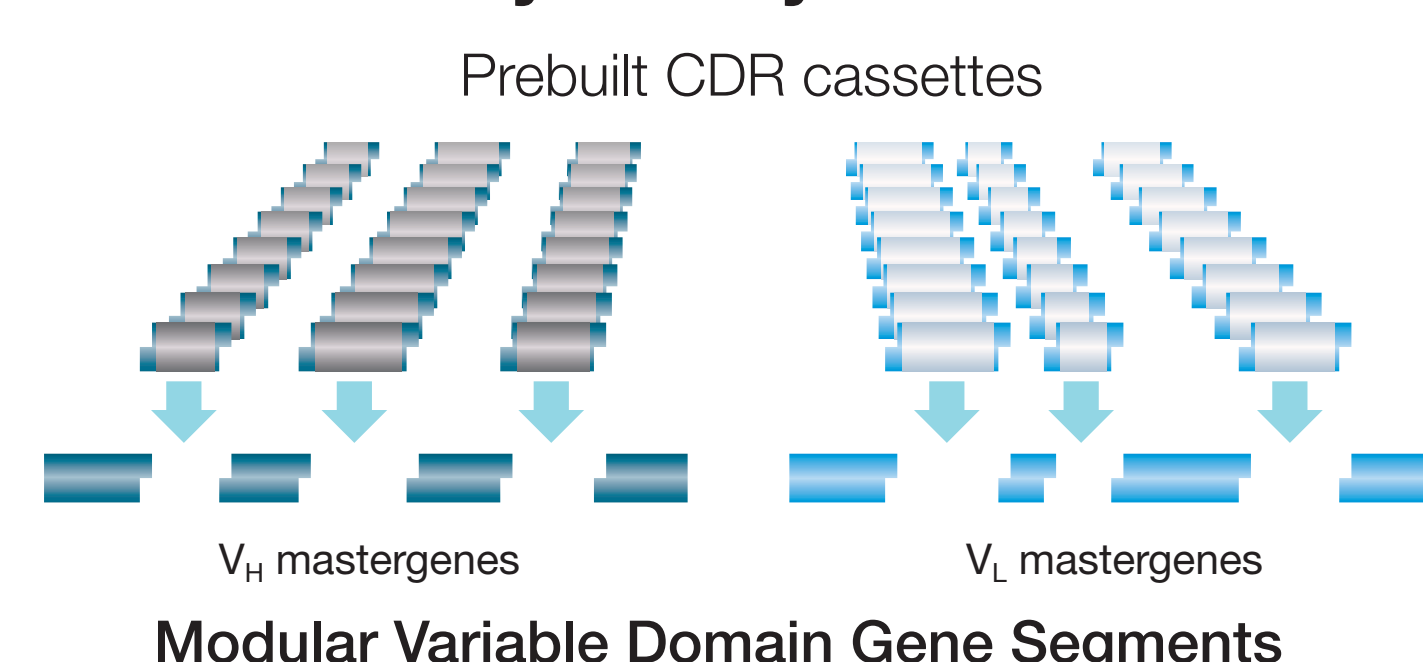


Results

Antibody	K_d [1/Ms]	k_d [1/s]	k_o [nM]
AbD23302	7.03E+06	1.53E-04	0.02
AbD23312	9.16E+05	2.24E-04	0.2

- Best antibody with affinity of 20 pM

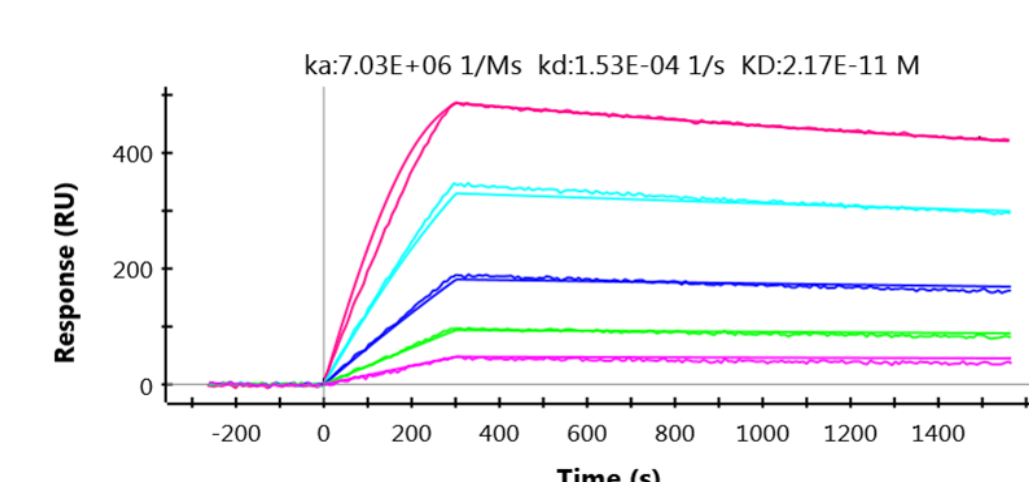
HuCAL PLATINUM Antibody Library



- Synthetic human antibody genes as building blocks, cover structural and sequence diversity in man
- Modular gene design for library generation, diversification by high-quality trinucleotide CDR cassettes, highly diverse repertoires in all six CDRs
- Initial library: 45 billion functional human antibodies in a test tube
- CysDisplay® - a proprietary phage display approach
- Optional affinity maturation using trinucleotide cassettes

Affinity Determination - Raw Data

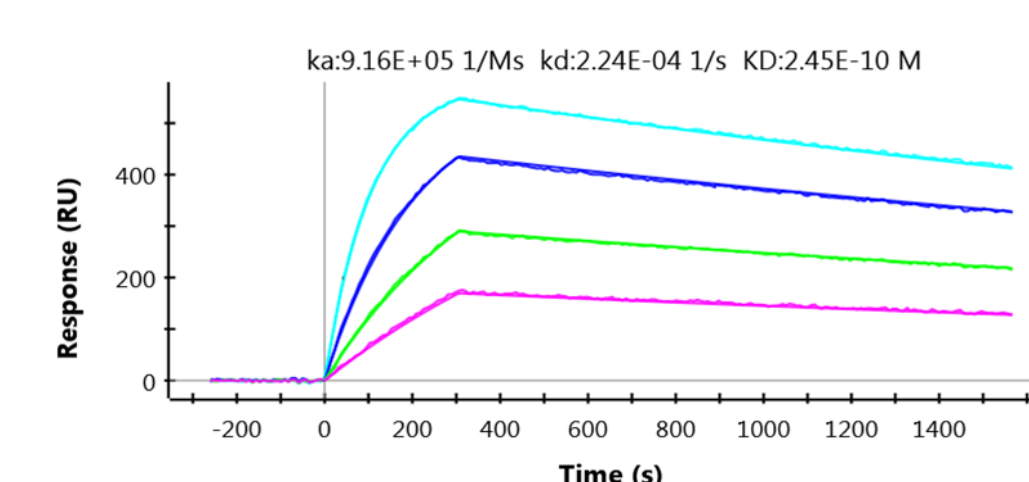
AbD23302 – Best antibody from panning on IL-6



Legend Sensorgrams:

- For AbD23302.1:
 - A1: 2.5 nM
 - A2: 1.25 nM
 - A3: 0.62 nM
 - A4: 0.31 nM
 - A5: 0.15 nM

AbD23312 – Best antibody from panning on mAb/IL-6 complex



For AbD23312:

- A1: 20 nM
- A2: 10 nM
- A3: 5 nM
- A4: 2.5 nM
- A5: 1.25 nM

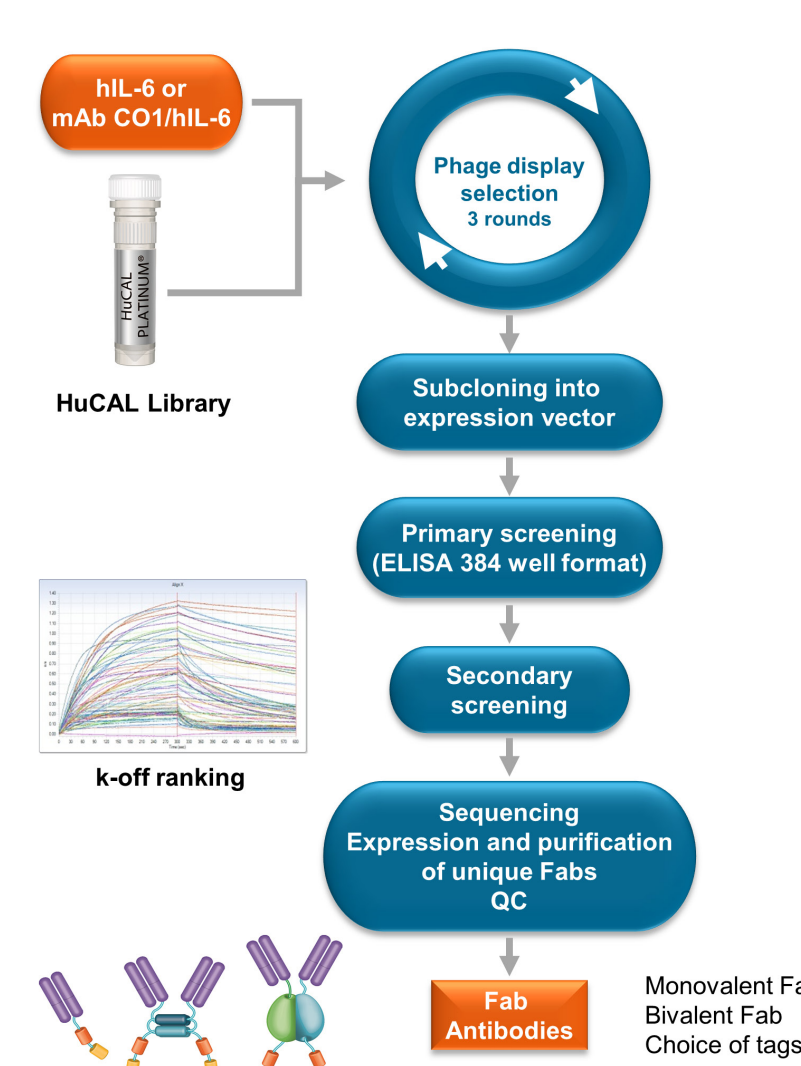
Objective and Project Overview

Objective: Generation of an antibody sandwich pair for human IL-6

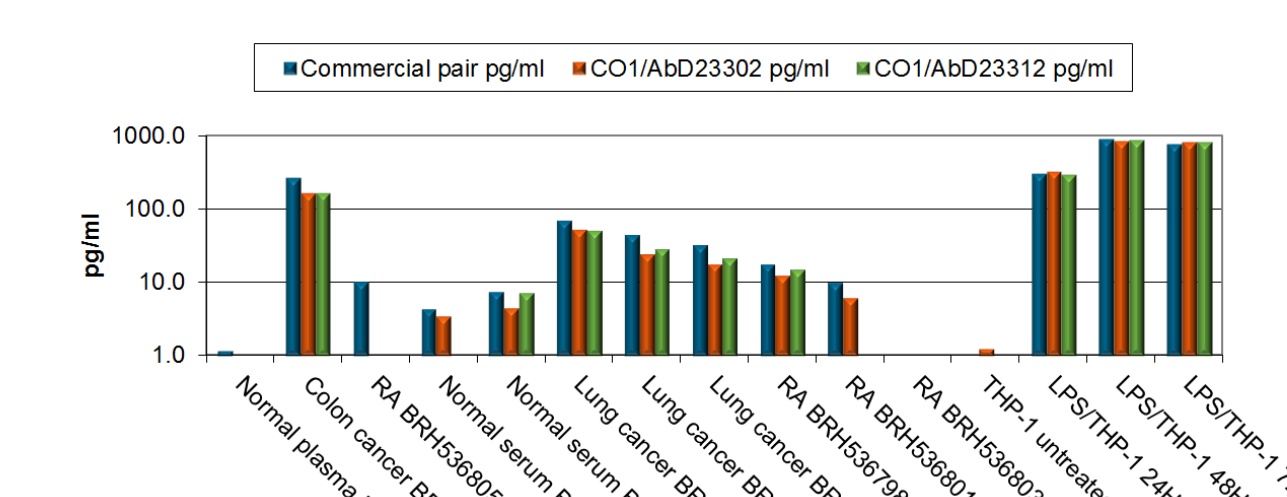
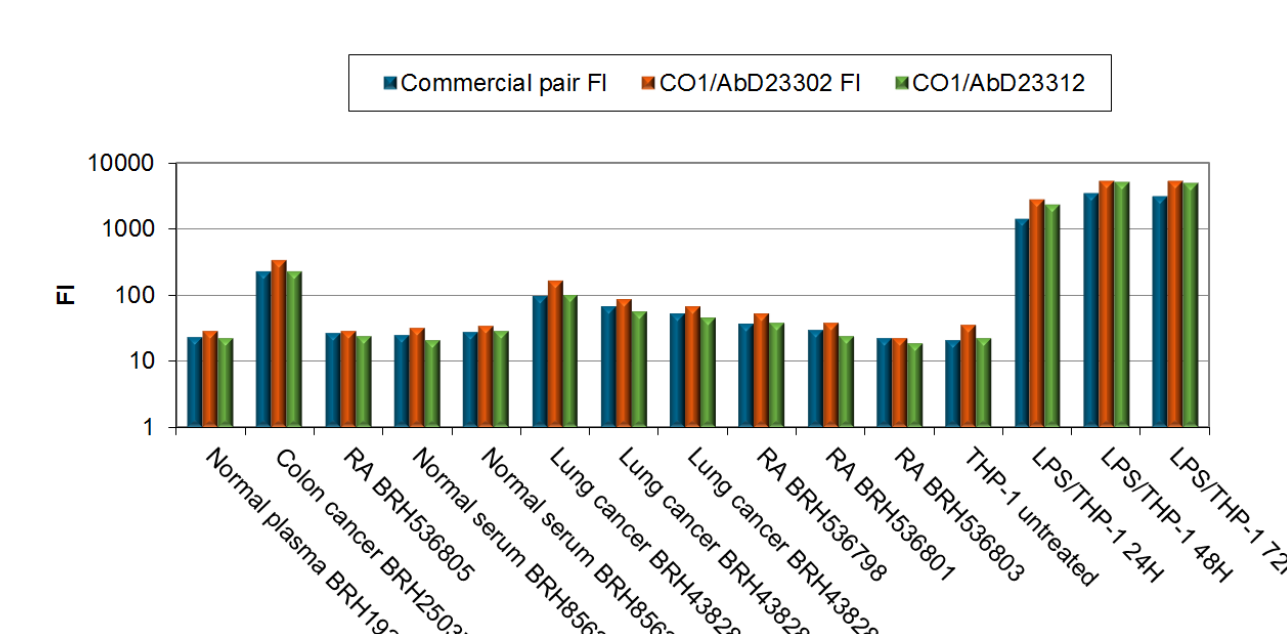
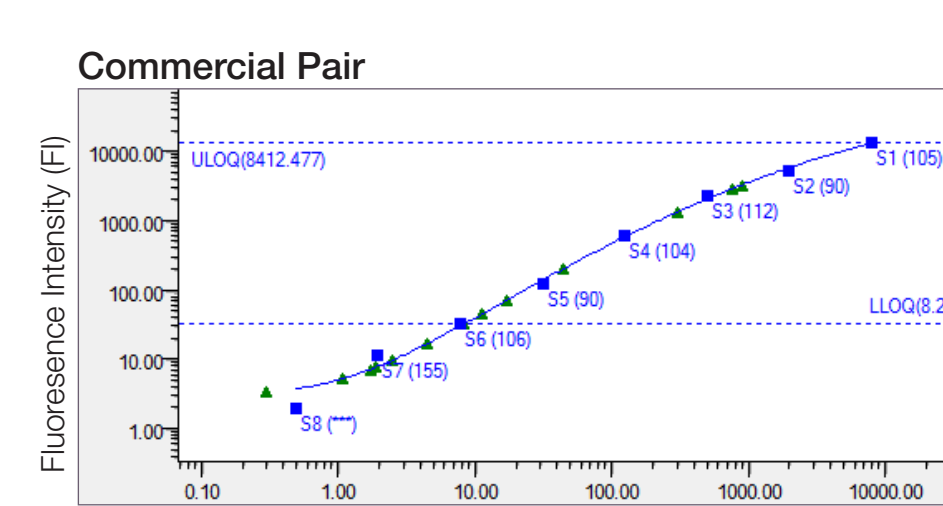
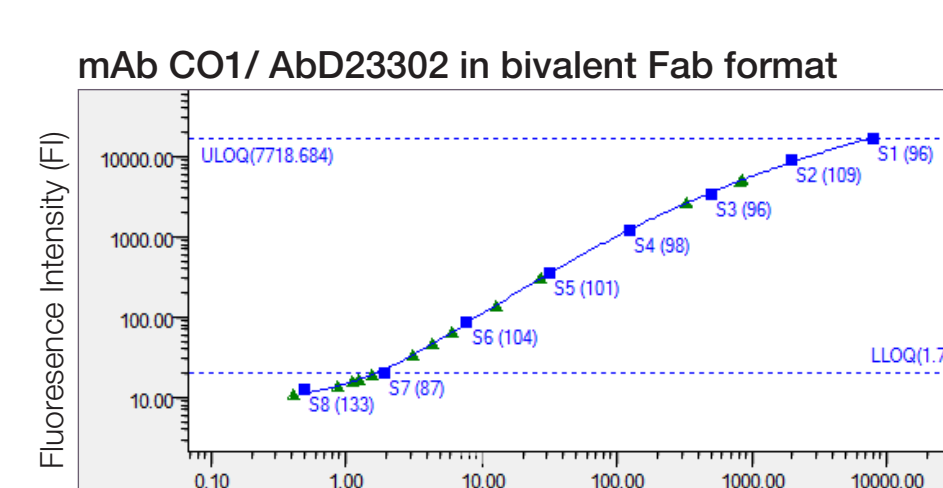
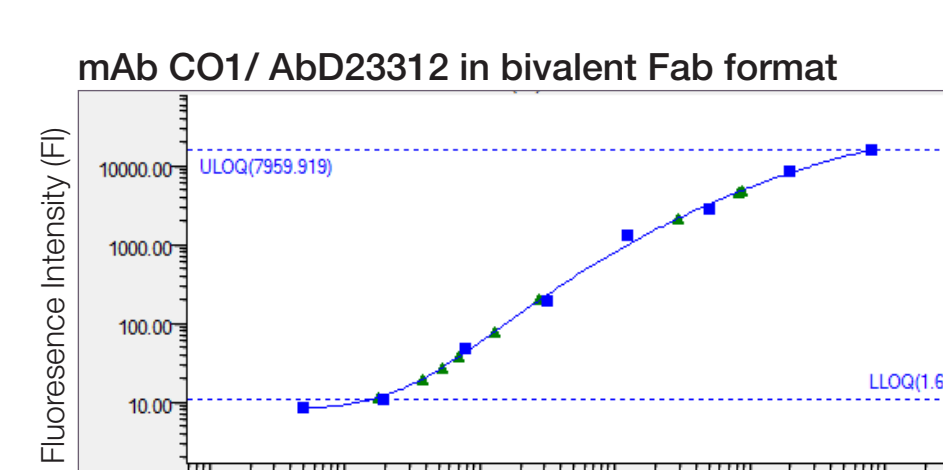
- Find new sandwich pair replacing existing hIL-6 antibodies in Bio-Plex panels
 - Good monoclonal capture antibody from Bio-Rad available
- No cross-reactivity with human serum or plasma
- Ideally no cross-reactivity with other cytokines
- Required sensitivity: <1 pg/ml

Project overview

- Panning strategies:
 - On immobilized recombinant IL-6
 - Capture panning on complex (Bio-Rad antibody CO1 / rec. IL-6)
- Pool subcloning into monovalent Fab-FH for primary ELISA screening
 - On rec. IL-6 and on CO1 / rec. IL-6 complex
- Secondary screening: k-off ranking
- Affinity determination
- Test in Bio-Plex sandwich assay using rec. hIL-6
- Conversion of best Fabs into bivalent Fab and hlgG1 format
- Test antibody in all formats in Bio-Plex sandwich assay with patient samples



Measuring IL-6 Sample Concentration in Bio-Plex



Performance of anti-IL-6 HuCAL antibodies in Bio-Plex

- The best antibody pair, consisting of mouse monoclonal antibody CO1 as capture and HuCAL antibody AbD23302 with 20 pM affinity to IL-6 as detection antibody, is more sensitive than the current antibody pair used in several Bio-Rad Bio-Plex panels
- All three antibody formats work well as detection antibody with mAb CO1
 - The bivalent format (Fab fused to alkaline phosphatase and hlgG1) are slightly better than the monovalent Fab
- Initial studies showed no cross reactivity with human cytokine panel I (27 targets)

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