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 affinity 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.

HuCAL PLATINUM Antibody Library

Modular Variable Domain Gene Segments
- 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

Objective and Project Overview
Objective: Generation of an antibody sandwich pair for human IL-6
- Find new sandwich pair replacing existing IL-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
- Planning 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. IL-6
  - Conversion of best Fab into bivalent Fab and Fh3G1 format
  - Test antibody in all formats in Bio-Plex sandwich assay with patient samples

Affinity Determination - Raw Data

Affinities of Best Antibodies
- Binding kinetics were measured on ProteOn XPR36 instrument
- Assay set-up:
  - For AbD23300 – 23310 are from panning on hIL-6 antibodies in Bio-Plex panels
  - A2: 1.25 nM
  - A4: 2.5 nM
- Optional affinity maturation using trinucleotide cassettes
  - AbD23300 – 23310 are from panning on hIL-6
  - A2: 10 nM
  - A4: 2.24E-04
  - Test in Bio-Plex sandwich assay using rec. hIL-6
  - 7.03E+06
- AbD23311 – 23314 are from panning on CO1/hIL-6 complex
  - Best antibody with affinity of 20 pM

Measuring IL-6 Sample Concentration in Bio-Plex

Affinities of Best Antibodies
- Binding kinetics were measured on ProteOn XPR36 instrument
- Assay set-up:
  - For AbD23311 – 23314 are from panning on CO1/hIL-6 complex
  - A5: 0.15 nM
  - A1: 2.5 nM
  - A3: 0.62 nM
  - A5: 1.25 nM
  - A1: 20 nM

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 hIgG1) are slightly better than the monovalent Fab
- Initial studies showed no cross-reactivity with human cytokine panel I (27 targets)

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

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