ProBioGen
Intelligent Biopharmaceutical Solutions

Cell-based Assay Services for Antibodies

At ProBioGen we offer a broad range of cell-based assays. These sophisticated in vitro assays are pivotal for the determination of product potency and for mode-of-action analyses in biopharmaceutical drug development. We establish new assays, optimize existing methods, screen your product candidates and validate these assays for batch-release.

Antibody-Dependent Cellular Cytotoxicity Assays

The establishment of cell-based activity assays for in-process controls and for API release-testing is an important step during drug development. One of these assays is the assessment of antibody-dependent cellular cytotoxicity (ADCC) mediated monoclonal antibodies.

Antibody-dependent cellular cytotoxicity (ADCC) is a mechanism of the immune system where an effector cell with cytolytic activity (e.g. NK cell) lyses a target cell that has been recognized by a specific antibody. *In vivo,* it is one of the mechanisms through which antibodies, as part of the humoral immune response, can act to limit and contain an infection. NK cell-mediated ADCC is triggered upon binding of the IgG's Fc region to the Fcγ receptor IIIA (Fcγ RIIIA, CD16a). Thus, NK cells specifically attack antibody-targeted virally infected or tumor cells, subsequently leading to their lysis. ADCC assays can be conducted with whole PBMCs or isolated primary NK cells to assess donor variability, and with stable CD16a-expressing cell lines.

The human FcγRIIIa on NK cells exists in two polymorphic forms at amino acid position 158 (phenylalanine or valine), which is located in the membrane-proximal IgG-binding domain. This variation results in binding of human IgG with higher affinity by NK cells of homozygous 158V/V donors than of homozygous 158F/F donors. Increased binding affinity of the receptor FcγRIIIa to an IgG molecule directly correlates with enhanced ADCC activity and, therefore, a more effective lysis of antibody-labeled target cells. ProBioGen AG also offers selection of homozygous 158F/F and V/V donors.

Advantages of ProBioGen's ADCC Assay:

- **Standardized NK cell separation from whole blood**
- **Genotyping of NK cell donors regarding the FcγRIIIa 158 V/F polymorphism**
- **Quality control of NK cells by flow cytometry (CD16/CD56 marker expression)**
- **Proprietary and fully characterized CD16-expressing NK cell line (in development)**
- **Established ADCC protocols for various antibody products (e.g. MabCampath®, Herceptin®, MabThera®)**
- **Comparative ADCC activity testing of different antibody clones (e.g. with modified glycosylation pattern)**
- **Different read-out parameters available**
- **Full logistic curve-fitting**
- **Direct cell lysis controls included in each assay (K-562 cells)**
- **ISO 9001/2008-certified regulatory environment**
- **FMEA-based risk assessment**
- **Validation of batch-release assays according to international guidelines**

Comparative ADCC assays determine the potency of antibody clones with different glycosylation patterns (*Figure 1).*

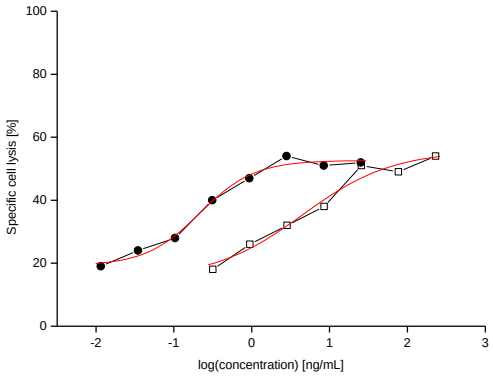

FIGURE 1: Comparison of GlymaxX® optimised Trastuzumab antibody (solid circles) with a trastuzumab WT antibody (open squares). The assay was conducted with primary NK cells and the BT-474 cell line.

Fcγ receptor binding assay

ProBioGen also established and qualified an Fcγ receptor binding assay, for characterizing the binding affinity of the Fc-part of a given antibody to the receptor FcγRIIIa. This assay can be used as a surrogate assay for screening of multiple antibodies (*Figure 2*). Antibody screening with both FcγRIIIa variants is possible.

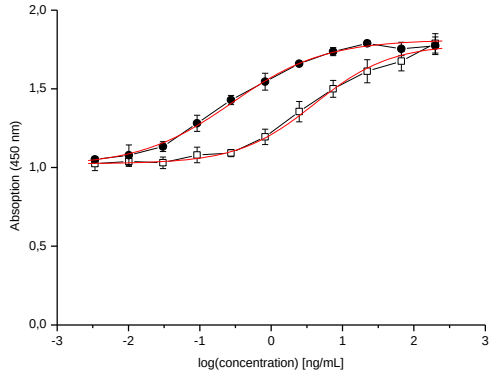

Figure 2: Binding curves of GlymaxX® optimized (solid circles) and WT IgG (open squares) to FcγRIIIa in the absence of plasma.

Additional *in vitro* antibody assays for the determination of product potency, functionality and mode-of-action analysis of the ProBioGen portfolio are:

- Complement-dependent cytotoxicity assays (CDC)
- Antibody-dependent cellular phagocytosis assay (ADCP)
- Ligand binding assays (ELISA format)