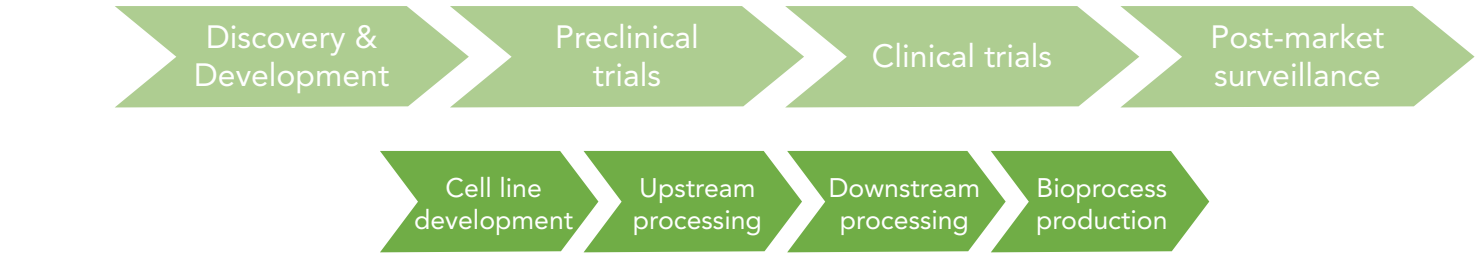
# IMMUNOASSAYS TO MAXIMIZE PRODUCTIVITY IN BIOPROCESS DEVELOPMENT




## THE BIOPHARMACEUTICAL CHALLENGE

The highly competitive and expanding market for biotherapeutics is being fueled by the success of existing antibody-based therapies, and the development of new therapies for the treatment of cancer and rare diseases. This puts great pressure on companies to reduce time to market while maintaining quality and maintain cost effectiveness in process development of antibody therapeutics.

High productivity demands efficient bioanalytical procedures including measuring product titer and process impurities, but commonly used methods, such as enzyme-linked immunosorbent assay (ELISA) or high-performance liquid chromatography, are time consuming and can create bottlenecks in critical workflows.

Immunoassays used to monitor bioprocess development should ideally possess these qualities to maximize efficiency:

- High quality data quickly delivered to support confident data-driven decision making
- Rapid assay development
- High analytical performance that is consistent between process stages
- Broad dynamic range and high matrix tolerance to cope with a diversity of samples, from expression to production
- Minimal demand on resources (hands-on-time and reagent consumption)
- High throughput when it counts e.g. in cell-line development and clonal selection
- Robustness for validation over long product life cycles to meet regulatory demands

## THE VALUE OF AUTOMATED NANOLITER SCALE IMMUNOASSAYS

Gyrolab systems meet the efficiency needs of many biopharmaceutical companies utilizing immunoassays to bioanalysis throughout bioprocess development. Gyrolab CD-based nanoliter scale immunoassay technology addresses bottlenecks and delays by providing rapid assay development time, automation, high throughput, and a broad dynamic range that minimizes dilutions and repeats.

Here we illustrate how Gyrolab immunoassays meet the critical requirements for immunoassays used in process development of an IgG therapeutic antibody, from initial cell culture to final purified product*, to measure:

- IgG product titer
- Host Cell Protein impurities
- Leached Protein A impurities

* Gyrolab assays have been used to follow the production and purification of a therapeutic antibody performed by Polymun GmbH. A CHO transfected cell line was cultured at 4-liter scale for 12 days. After cell culture, the therapeutic antibody of IgG1 subtype was purified by two-step chromatography involving affinity purification on MabSelect SuRe™ LX and flow-through Anion EXchange chromatography (AEX). Samples for analysis were collected during cell culture and throughout the purification process. Gyros Protein Technologies wishes to thank POLYMUN Scientific Immunbiologische Forschung GmbH for generously supplying the samples for analysis.

# IgG QUANTIFICATION

Product titer assays to determine monoclonal antibody concentration during biopharmaceutical development must accurately quantify titers over a broad concentration range. In cell line development and clone selection, titers can vary greatly and sample numbers may be high, whereas in downstream process development concentrations are high and must be measured with high analytical precision and reproducibility.

## HIGH THROUGHPUT IN CELL LINE DEVELOPMENT AND CLONAL SELECTION

Decisions on the cell line and final clone selection to are typically made early since changes to cell line are considered major and require comparability studies. Rapid screening and timely selection of highly productive and stable clones are major challenges.

Gyrolab immunoassays deliver the robustness, rapid turnaround time, and high throughput needed for efficient screening. Figure 1 shows titer distribution of clones in a screen of low titers during early cell line development. Post-transfection samples were measured for IgG titer using Gyrolab xP workstation, which processed 332 samples in four hours (data kindly supplied by Merck).

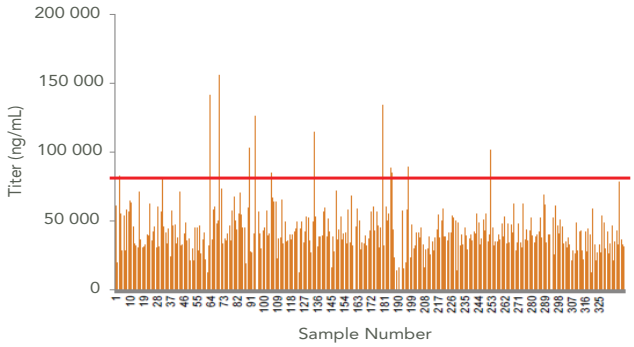



***Figure 1.*** *Early IgG titer distribution of clones determined using Gyrolab xP workstation*

## IgG TITER IN CELL CULTURE

IgG titer varies greatly during cell culture. In our example, the IgG titer throughout the 12-day cell culture was robustly measured using the Gyrolab assay run on both Gyrolab xPlore and Gyrolab xP workstation. The data correlated well with data from an ELISA.

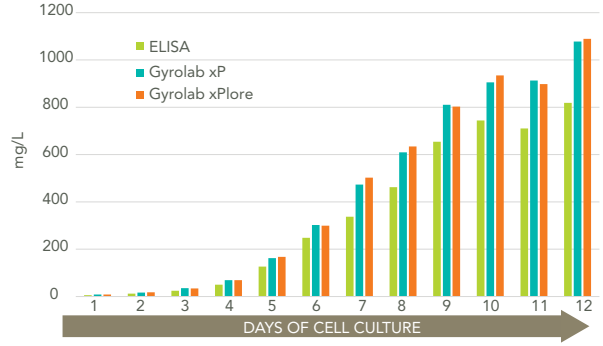



**Figure 2.** *IgG titer immunoassay in 12-day cell culture*

## IgG TITER DURING PURIFICATION PROCESS STEPS

The Gyrolab assay was then be used to measure the high IgG titers during the two-step purification of the IgG biologic from harvested cells. Overall, there was good correlation between IgG concentration data from Gyrolab xPlore, ELISA and $OD_{280}$ (typically used in later process stages to measure titer).

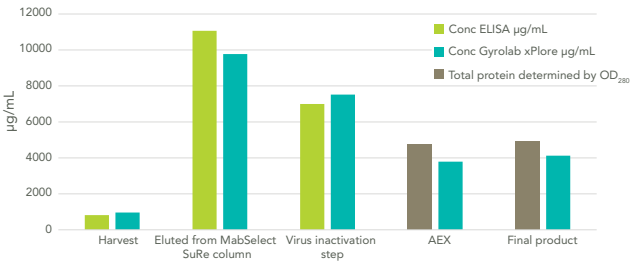



***Figure 3.*** *Titer of therapeutic IgG during purification steps*

## GYROLAB KITS FOR DETERMINATION OF HUIGG

Gyros Protein Technologies has developed two kits that rapidly determine human IgG over a combined range of six logs. Gyrolab systems completely automate the analytical process and generate 96 or 112 datapoints in approximately one hour. The kits are designed to quantify intact human IgG1, IgG2 and IgG4 in cell cultures.

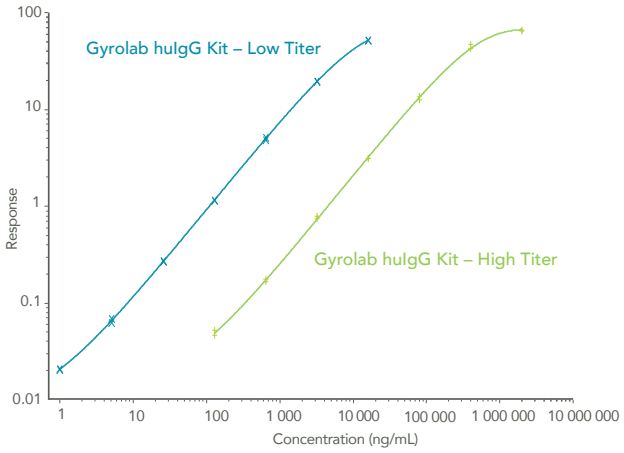



***Figure 4.*** *Standard curves from Gyrolab huIgG Low and High Titer Kits*

## SUMMARY

Gyrolab huIgG kits for quantifying IgG titer deliver:

- Automated and rapid analyses delivering 96 datapoints in 1 hour to meet the needs of screening and rapid data-driven decision making
- Broad dynamic range, minimizing the need for dilutions and maximizing coverage of samples, from cell line development/clonal selection, through cell culture, to IgG purification

# HOST CELL PROTEIN IMPURITIES

Ensuring minimal levels of Host Cell Protein (HCP) impurities from expression systems is critical during bioprocess development of recombinant therapeutic antibodies. HCPs in biotherapeutics are potentially immunogenic and therefore regulated. Residual HCP is a complex protein mixture that is routinely measured using ELISA with polyclonal antibodies, but ELISA suffers from narrow dynamic range and long turnaround times, and requires frequent manual interventions and repeat analysis.

## PRECISE DETERMINATION OF HCP IMPURITIES

In their search for more robust methods for HCP analysis, MedImmune compared an ELISA with a Gyrolab immunoassay in the analysis of HCP in samples from different stages in the purification of a therapeutic protein. They found that there was excellent correlation of results from the two methods, and that the Gyrolab assay improved dilutional linearity as a result of broader dynamic ranges and good precision, enabling them to make rapid data-driven decisions.

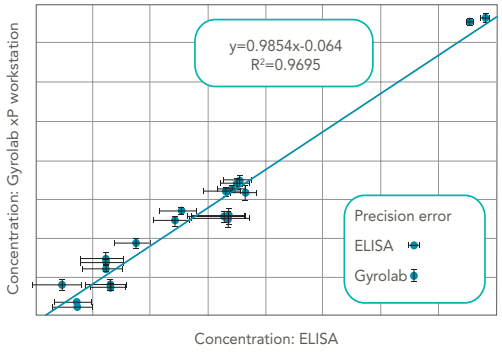



***Figure 5.*** *Correlation of ELISA and Gyrolab immunoassay HCP analysis*

## CHO-HCP ASSAY WITH BROAD DYNAMIC RANGE

To address many of the limitations of ELISA, Gyros Protein Technolgies has developed a sandwich immunoassay kit specific for the detection of HCP impurities from Chinese Hamster Ovary (CHO), the cell line most commonly used in the production of therapeutic antibodies.

Gyrolab CHO-HCP Kit 1 was used to analyze samples from a purification process performed at Polymun Scientific. The assay delivered excellent linearity over a four-log range, compared to only two logs for ELISA (Figure 6) and higher signal/background (S/B) ratio for low concentrations. The broad dynamic range simplified spike recovery, dilution linearity experiments, and reduced the risk of repeat analysis.

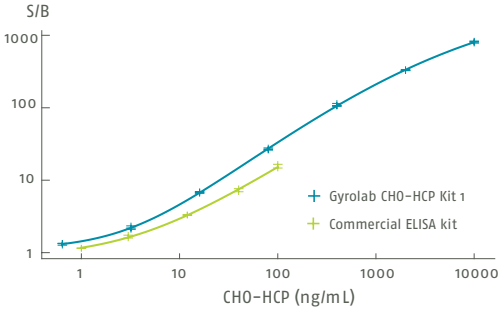



***Figure 6.*** *Dynamic range of Gyrolab CHO-HCP Kit 1 compared to ELISA*

The assay automation on Gyrolab system enabled 96 data points to be generated in approximately 75 minutes without manual intervention, and with minimal hands-on time.

| | Commercial ELISA | Gyrolab automated systems |
|---|---|---|
| Dynamic range | 1 -100 ng/mL | 1 - 8000 ng/mL |
| LLOQ (sensitivity) | 1 ng/mL | 1 ng/mL |
| LOD (limit of detection) | 0.3 ng/mL | 0.64 ng/mL |
| Sample requirements | 50 µL | 4 µL |
| Sample preparation | 60 min | 60 min |
| Assay run time | 150 min with several manual interventions | 75 min automated run |
| Total assay time | 4 hrs 15 min | 2 hrs 15 min |
| Throughput (day) | 5 plates/day manual | up to 10 CDs/day automated |
| Data points | 96 wells per plate | 96 microstructures per CD |
| Overnight run | No | Yes |

## RAPID CHO-HCP ANALYSIS OF PURIFICATION SAMPLES

Gyrolab CHO-HCP Kit 1 was used to measure HCP in selected samples from the IgG1 therapeutic antibody purification at Polymun. The results were comparable to those from a commercial ELISA kit. The automated workflow of the Gyrolab system delivered the results in 1 hour, in contrast to ELISA, which demanded 3–4 hours and manual interventions.

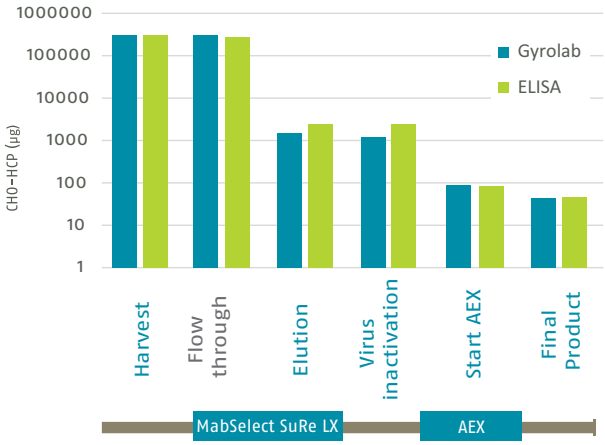



***Figure 7.*** *HCP quantitation of bioprocess purification samples*

## SUMMARY

Gyrolab CHO-HCP Kit 1 run on Gyrolab instruments delivers:

- Assay robustness, convenience and rapid results
- Broad dynamic range — over four logs that minimizes dilutions and repeats
- Automation and efficient analysis that reproducibly delivers 96 data points in 75 min, with minimal hands-on time and risk for error

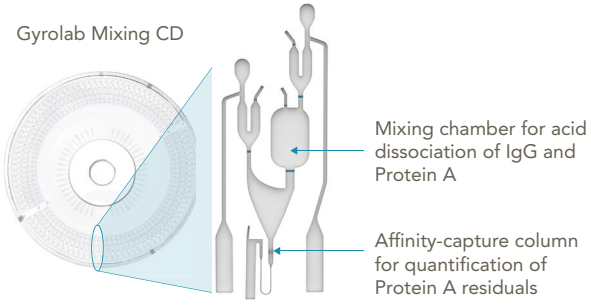
# LEACHED PROTEIN A IMPURITIES

Protein A binds to human IgG, and is invaluable in affinity chromatography for the purification of therapeutic antibodies. Protein A can, however, leach from the chromatography resin and co-elute with the therapeutic antibody product. Protein A may bind to immunoglobulins, lead to adverse reactions, and is regarded as a process impurity that must be monitored. It is therefore essential to accurately quantify Protein A during the purification process, and prior to product release.

## STREAMLINING SAMPLE PREPARATION WITH AUTOMATED ACID DISSOCIATION

Measuring Protein A or Protein A-derived ligands directly in bioprocess samples may underestimate the contamination level since IgG binds to relevant Protein A epitopes, preventing accurate Protein A quantification. Sample preparation must therefore include dissociation of Protein A and IgG, increasing the complexity of the assay workflow.

The Gyros method for quantifying residual Protein A ligands includes convenient and robust automated sample dissociation. Samples are treated with a low pH buffer in Gyrolab Mixing CD to dissociate the IgG product and Protein A, which is measured using a sandwich immunoassay in the same CD.




The assay workflow for the automated analysis of leached Protein A-derived ligands was greatly simplified compared to ELISA.

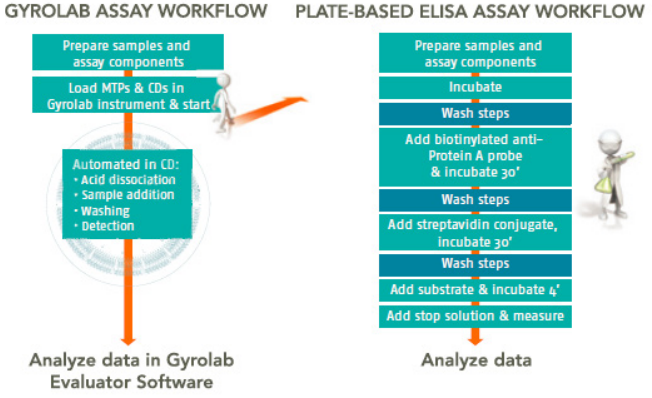



***Figure 8.*** *Assay workflow comparison: Gyrolab and ELISA*

The Gyrolab assay delivers excellent linearity and reproducibility, in this case with intra-assay Coefficients of Variation (CV) for duplicates in standard curves of 7.6 % or better, and inter-assay CVs for duplicates of control samples run on three CDs, of 5.3 % or better.

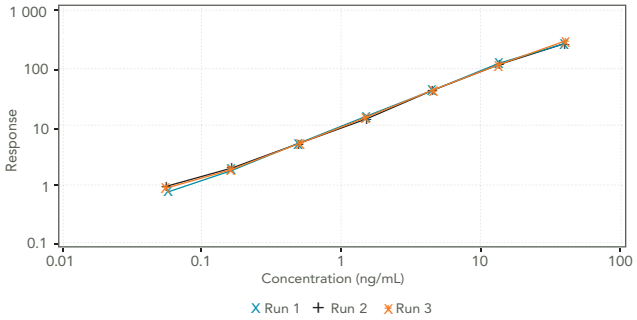



***Figure 9.*** *Gyrolab Protein A standard curves run on 3 CDs*

## MEASUREMENT IN PURIFICATION SAMPLE

The samples from the purification using MabSelect SuRe LX, a Protein A-derived ligand, were analyzed using Gyrolab Protein A kit and Repligen's Protein A ELISA kit, run according to the protocol for the Dilute & Go method. Samples were measured in duplicate (Gyrolab) or triplicate (Repligen), giving CVs of <10%.

| Sample | IgG (mg/mL) | MabSelect SuRe LX | | | |
|---|---|---|---|---|---|
| | | Concentration (ng/mL) | | MabSelect SuRe LX/IgG (ppm) | |
| | | Gyrolab Protein A Kit* | Repligen Dilute & Go** | Gyrolab Protein A kit | Repligen Dilute & Go |
| Elution from MabSelect SuRe LX column | 9.8 | 3.0 | 3.3 | 3.0 | 6.5 |
| Virus Inactivation | 7.5 | 1.7 | 1.6 | 1.7 | 3.1 |
| Start AEX | 4.4 | 0.1 | 0.1 | 0.1 | 0.1 |
| Flow through and wash from AEX column | 3.8 | 0.1 | 0.1 | 0.1 | 0.1 |
| Final Product | 4.1 | 0.1 | 0.1 | 0.1 | 0.1 |

* Samples diluted to 1 mg/mL IgG  ** Samples diluted to 0.5 mg/mL IgG

*The results showed comparable IgG quantitation data from the two methods and confirmed the efficient removal of leached MabSelect SuRe LX ligand during purification.*

## SUMMARY

Gyrolab Protein A kit for measurement of Protein A impurities delivers:

- Quantification of residual native Protein A, recombinant Protein A variants and MabSelect SuRe
- High quality data delivered within 80 minutes, with high sensitivity, precision, accuracy and reproducibility
- Major workflow benefits over ELISA, with automated acid dissociation, minimized manual steps, hands-on time and risk for error



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