

# Gyrolab® hulgG Kits

## Product Information Sheet

D0024959/H

- **Easy to use**
  - Optimized kit ensures assay robustness, convenience and consistency of results
  - No need for assay development
- **Broad dynamic range and robustness**
  - Dynamic range of 3–4 logs for each kit, with sensitivity down to 1 ng/mL
  - Reduced need for dilution steps
- **Automated and rapid analysis supporting data driven decisions**
  - 96 data points in 1 hour
  - Reduced hands-on operations and risk for errors
  - Reproducible high quality data
  - Unattended run generates up to 560 data points

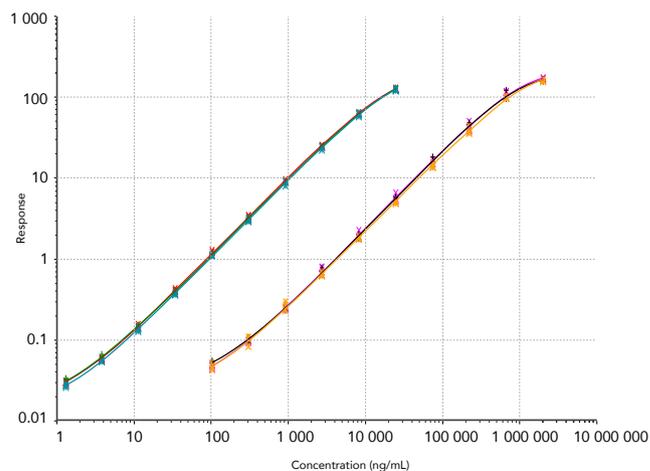


## Introduction

The development and manufacture of biotherapeutics requires efficient and timely analytical support to monitor quantity and quality in products and processes. Productivity is important already during early development. Further downstream, selected cell lines must be optimized for productivity, followed by bioprocess development to yield g/L concentrations of IgG product.

Immunoassays are often used to quantify IgG titers to provide critical information for efficient decision making. Such assays must have the flexibility needed to measure the broad range of product concentrations that are encountered during cell-line and process development.

To meet these needs, Gyros Protein Technologies has developed two kits to rapidly determine human intact IgG titers that together cover a range of six logs (Figure 1). The kits are composed of ready-to-use reagents and are compatible with two Gyrolab® Bioaffy CDs that differ in sample volume. The concentration range of each assay, typically 3-4 logs, depends on the CD used - Gyrolab Bioaffy 1000 HC for low titers and Gyrolab Bioaffy 20 HC for high titers. Gyrolab systems completely automate the analytical process and generate 96 or 112 data-points in approximately one hour. The kits are designed to quantify human IgG1, IgG2 and IgG4 in cell cultures.



**Figure 1.** Gyrolab hulgG Kit – Low Titer and Gyrolab hulgG Kit – High Titer provide reliable IgG titer measurements over a broad dynamic concentration and with excellent reproducibility. The figure shows overlaid standard curves from three runs of each kit.

## Assay characteristics

Gyrolab hulG Kits have been developed to quantify intact human IgG (IgG1, IgG2, IgG4) in cell supernatants during cell line development. Gyrolab hulG Kits add convenience and also significant workflow benefits compared to ELISA: the kits cover a broad concentration range with reduced matrix interference, and the automation provided by the Gyrolab systems significantly reduce the number of manual steps, risk for error, and time to results.

**Table 1.** Two configurations of Gyrolab hulG Kit

| Kit                           | Contents                      | Size   | LOD       | LLOQ      | ULOQ     |
|-------------------------------|-------------------------------|--------|-----------|-----------|----------|
| Gyrolab hulG Kit – High Titer | Reagent Kit + Bioaffy 20 HC   | 112 dp | 100 ng/mL | 300 ng/mL | 1 mg/mL  |
| Gyrolab hulG Kit – Low Titer  | Reagent Kit + Bioaffy 1000 HC | 96 dp  | 0.5 ng/mL | 1 ng/mL   | 20 µg/mL |

Combining the same reagent kit with two different Bioaffy CDs enables analysis of samples with a large range of concentrations, from ng/mL to mg/mL. If samples contain low concentrations of IgG, Gyrolab hulG Kit – Low Titer should be selected. If concentrations in samples are high then Gyrolab hulG Kit – High Titer should be used. See table 1.

The flow-through, affinity sandwich immunoassay is based on the biotinylated Fz fragment as capturing molecule, which is specific for the Fc portion of human intact IgG. An anti-F(ab')<sub>2</sub> antibody is used as detection antibody.

The biotinylated reagent is introduced into a microstructure in the CDs to saturate an affinity-capture column packed with porous beads that are coupled with streptavidin. Subsequently, cell supernatant or bioprocess samples containing intact human IgG are volume-defined and introduced into the microstructures where intact IgG binds onto the affinity column and the remaining matrix components are washed away. Finally, a detection reagent labeled with a suitable fluorophore is added.

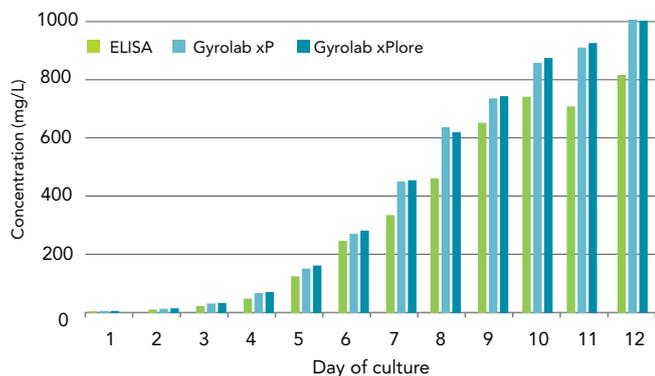
Gyrolab hulG Kits do not contain standard, but this can be ordered as a separate product from Gyros Protein Technologies.

## High reproducibility and broad dynamic range

Figure 2 shows the results from a series of samples, taken daily over twelve days from a cell culture producing an IgG therapeutic antibody. The samples were diluted 1:2 and analyzed using Gyrolab hulG Kit – High Titer in six runs on two Gyrolab instrument models and the results were compared with those determined with an ELISA. Figure 3 demonstrates that the concentrations determined with the kit on the two Gyrolab instruments correlate well with concentrations obtained from an ELISA. Table 2 shows the intra- and inter-precision data for the two Gyrolab instruments which in most cases demonstrated CV's <10%.

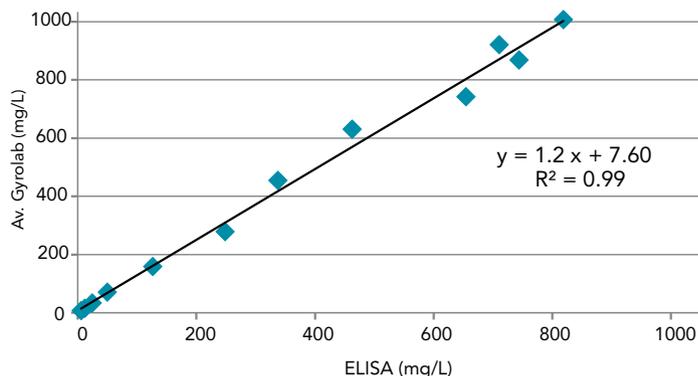
**Table 2.** Data comparison between different Gyrolab instruments and an ELISA for a series of cell culture samples.

|           | Gyrolab xP workstation |                       |                       | Gyrolab xPlore        |                       |                       | ELISA             |
|-----------|------------------------|-----------------------|-----------------------|-----------------------|-----------------------|-----------------------|-------------------|
|           | Av conc (mg/L)<br>n=9  | Intra run % CV<br>n=3 | Inter run % CV<br>n=9 | Av conc (mg/L)<br>n=9 | Intra run % CV<br>n=3 | Inter run % CV<br>n=9 | ELISA conc (mg/L) |
| Sample 1  | 7.2                    | 3                     | 7                     | 7.7                   | 7                     | 11                    | 5.9               |
| Sample 2  | 15.0                   | 2                     | 6                     | 16.1                  | 10                    | 13                    | 12.2              |
| Sample 3  | 33.2                   | 13                    | 11                    | 34.7                  | 7                     | 11                    | 24.5              |
| Sample 4  | 69.2                   | 10                    | 11                    | 72.7                  | 8                     | 10                    | 49.9              |
| Sample 5  | 153.4                  | 9                     | 9                     | 163.5                 | 6                     | 9                     | 126.4             |
| Sample 6  | 272.5                  | 8                     | 10                    | 283.9                 | 9                     | 10                    | 248.4             |
| Sample 7  | 452.4                  | 10                    | 10                    | 456.5                 | 7                     | 7                     | 337.2             |
| Sample 8  | 639.3                  | 9                     | 8                     | 621.7                 | 1                     | 5                     | 462.3             |
| Sample 9  | 737.9                  | 2                     | 4                     | 745.6                 | 2                     | 4                     | 654.4             |
| Sample 10 | 860.5                  | 2                     | 5                     | 876.7                 | 2                     | 4                     | 744               |
| Sample 11 | 913.1                  | 3                     | 6                     | 928.3                 | 4                     | 4                     | 710.5             |
| Sample 12 | 1 009.2                | 2                     | 4                     | 1 004.7               | 3                     | 3                     | 818.5             |



**Figure 2.** The IgG titer throughout the 12-day cell culture was measured with Gyrolab hulG Kit - High Titer and the results correlate well with data from ELISA.

Table 3 shows twelve samples covering a large concentration range analyzed using both kits. In this example, Gyrolab hulG Kit – Low Titer measured samples with concentrations from 2.4 ng/mL to 6.5 µg/mL, while Gyrolab hulG Kit – High Titer measured samples with concentrations from 219 ng/mL to 0.88 mg/mL. An overlapping range was obtained between 220 ng/mL and 7 µg/mL with excellent correlation between kits.



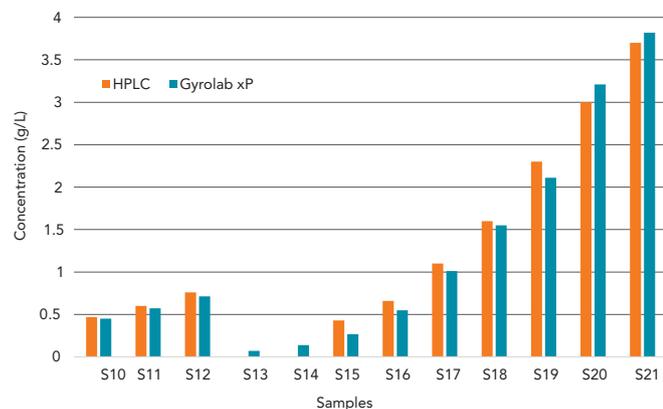
**Figure 3.** Results from Gyrolab hulG Kit (average of concentration from each Gyrolab model) correlate well with those from ELISA.

**Table 3.** A series of bioprocess samples containing low to high IgG titers were analyzed using both kits

|           | Gyrolab hulG - Low Titer Conc. (ng/mL) | Gyrolab hulG - High Titer Conc. (ng/mL) |
|-----------|--|---|
| Sample 1  | 2.4                                    | <LLOQ                                   |
| Sample 2  | 7.0                                    | <LLOQ                                   |
| Sample 3  | 23                                     | <LLOQ                                   |
| Sample 4  | 72                                     | <LLOQ                                   |
| Sample 5  | 226                                    | 219                                     |
| Sample 6  | 671                                    | 747                                     |
| Sample 7  | 1 930                                  | 2 350                                   |
| Sample 8  | 6 474                                  | 7 000                                   |
| Sample 9  | >ULOQ                                  | 21 100                                  |
| Sample 10 | >ULOQ                                  | 70 503                                  |
| Sample 11 | >ULOQ                                  | 233 800                                 |
| Sample 12 | >ULOQ                                  | 882 600                                 |

## Comparison of data generated with Gyrolab hulG Kit and protein A based HPLC

Concentration data from Gyrolab hulG Kits and HPLC are comparable. Figure 4 and Table 4 show the IgG concentrations of a series of cell culture samples as determined with HPLC using a Protein A column and Gyrolab hulG Kit – High Titer. The standard in both assays was project specific. For samples 01-09 the sensitivity of the HPLC method was insufficient while the Gyrolab kit determined concentrations down to 5.5 µg/mL with excellent precision.



**Figure 4.** Excellent correlation between IgG concentrations determined with Gyrolab hulG Kit and HPLC. Samples 01 - 09 could not be quantified with the HPLC method. Data kindly provided by Cobra Biologics, Sweden

**Table 4.** Comparison of IgG concentration data measured with HPLC and Gyrolab hulG - High Titer Kit

|           | HPLC (g/L) | Gyrolab hulG kit- High Titer (g/L) | Gyrolab xP CV% n=2 |
|-----------|------------|------------------------------------|--------------------|
| Sample 1  | <0.41      | 0.0055                             | 2.6                |
| Sample 2  | <0.41      | 0.0086                             | 0.2                |
| Sample 3  | <0.41      | 0.0171                             | 0.4                |
| Sample 4  | <0.41      | 0.0297                             | 0.6                |
| Sample 5  | <0.41      | 0.0558                             | 2.7                |
| Sample 6  | <0.41      | 0.0966                             | 0.9                |
| Sample 7  | <0.41      | 0.1730                             | 0.1                |
| Sample 8  | <0.41      | 0.2450                             | 1.3                |
| Sample 9  | <0.41      | 0.3310                             | 6.3                |
| Sample 10 | 0.47       | 0.4520                             | 0.7                |
| Sample 11 | 0.60       | 0.5720                             | 1.4                |
| Sample 12 | 0.76       | 0.7140                             | 2.0                |
| Sample 13 | <0.38      | 0.0706                             | 0.4                |
| Sample 14 | <0.38      | 0.1400                             | 1.0                |
| Sample 15 | 0.43       | 0.2680                             | 0.5                |
| Sample 16 | 0.66       | 0.5490                             | 0.6                |
| Sample 17 | 1.1        | 1.0100                             | 0.5                |
| Sample 18 | 1.6        | 1.5500                             | 1.0                |
| Sample 19 | 2.3        | 2.1100                             | 0.5                |
| Sample 20 | 3          | 3.2100                             | 2.7                |
| Sample 21 | 3.7        | 3.8200                             | 0.1                |

## Ordering Information

| Product number | Product name                                    |
|----------------|---|
| P0020382       | Gyrolab hulG Kit - High Titer (112 data points) |
| P0020381       | Gyrolab hulG Kit - Low Titer (96 data points)   |
| P0020379       | Gyrolab hulG Standard (IgG1)                    |

| Gyrolab hulG Kit contents   | Quantity  |
|---|-----------|
| Gyrolab hulG Kit Reagents (contents see below)                            | 1 of each |
| Gyrolab Bioaffy 1000 HC (Low Titer) or Gyrolab Bioaffy 20 HC (High Titer) | 1         |
| PCR plate 96  | 3         |
| Microplate foil   | 3         |
| Gyrolab Wash Buffer pH 11   | 1         |

### Gyrolab hulG Kit Reagents

- Reagent A** Capture Reagent, Biotinylated derivative of protein A from *Staphylococcus aureus*. Ready to use solution, 60 µL
- Reagent B** Detection Reagent, Alexa Fluor 647 labeled F(ab')<sub>2</sub> fragment of anti-human IgG. Ready to use solution, 60 µL
- Reagent C** Wash Buffer 1, 1.5 mL
- Reagent D** Wash Buffer 2, 1.5 mL
- Reagent E** Sample Dilution Buffer, 25 mL

### Gyrolab hulG Standard

Standard containing intact IgG1 is provided as a separate product; 50 µL at 4 mg/mL.

### Storage conditions

#### Gyrolab Bioaffy 1000 HC CD and Gyrolab Bioaffy 20 HC CD

Refrigerate at +4 °C to +8°C, unopened package.

Shelf life (unopened package): see product label

#### Gyrolab hulG Kit reagents

Refrigerate at +4°C to +8°C. Do not freeze.

Shelf life (unopened package): see product label.

### Related products

Scan the QR-code to learn more about our other ready-to-use kits and solutions used for bioprocess analytics:



Gyrolab and Rexpip are registered trademarks and Gyros, Gyrolab xPlore, Gyroplex, Bioaffy and Gyros logo are trademarks of Gyros Protein Technologies Group. All other trademarks are the property of their respective owners. Products and technologies from Gyros Protein Technologies are covered by one or more patents and/or proprietary intellectual property rights. All infringements are prohibited and will be prosecuted. Please contact Gyros Protein Technologies AB for further details. Products are for research use only. Not for use in diagnostic procedures. © All infringements are prohibited and will be prosecuted. Please contact Gyros Protein Technologies AB for further details. Products are for research use only. Not for use in diagnostic procedures. © Gyros Protein Technologies AB 2022.