Instruction For Use

Gyrolab® huIgG Kit – High Titer
Gyrolab® huIgG Kit – Low Titer

Product number	Product Name
P0020382	Gyrolab huIgG Kit – High Titer
P0020381	Gyrolab huIgG Kit – Low Titer
P0020379	Gyrolab huIgG Standard (IgG1)

1. Intended use

This document describes a protocol to quantify intact human IgG (IgG1, IgG2, IgG4) in cell supernatants and downstream processes of therapeutic antibodies on Gyrolab immunoassay platforms. The ready-made reagents are provided in a kit format to be used together with one of two CDs, Bioaffy 20 HC or Bioaffy 1000 HC. These CDs use different sample volumes and therefore provide different working ranges of the assays according to needs. When combined, the kit will enable quantification of human IgG over six orders of magnitude.

Gyrolab® huIgG Kit is for research use only and not intended for diagnostic use.

2. Introduction

During the last decades, recombinant therapeutic antibodies have been established as key to the treatment of autoimmune disorder, cancer and infection. The relative success of these new drugs has stimulated the development of new candidates that are evaluated in clinical studies for desired and adverse effects in patient groups. Drug development requires several analytical methods to support efficient product development and lower risk in decision-making. These methods include IgG titer determination to identify IgG-producing clones or to assess the outcome of cell line or cell culture optimization.

3. Assay description

Gyros Protein Technologies has developed a kit to quantify intact human IgG (IgG1, IgG2, IgG4) in cell supernatant. The kit is based on a set of reagents that are used in combination with:

- Bioaffy 20 HC, for samples containing high human IgG titers
- Bioaffy 1000 HC, for samples containing low human IgG titers

A biotinylated reagent is introduced into a microstructure in the CD to saturate a capture column packed with porous beads coupled with streptavidin. Cell supernatant or bioprocess samples containing intact human IgG are then volume-defined and introduced into the microstructures where intact IgG is captured in the capture column. Finally, a detecting reagent labeled with a suitable fluorophore is added. The integrated signal in the capture column represents the total response from the sample.

4. Limitations

Partial Use of Kits

For single CD kits we recommend no more than two times partial use of kit reagents and CD that have been removed from its pouch.

Assay qualification

The Gyrolab huIgG Kit is intended for quantification of intact human monoclonal IgG in cell culture or bioprocess samples. The Gyrolab huIgG Kit is compatible with monoclonal IgG1, IgG2 and IgG4. We recommend that you qualify the assay for its intended use to ensure that the assay protocol provides acceptable performance, such as quantifiable range, accuracy, and precision.

5. Storage and Stability

Reagents

Gyrolab huIgG Reagent Kit must be stored at +4 to +8°C to maintain functionality. Gyrolab Wash Buffer pH 11 can be stored at +4 to+28°C.

Unopened CD package

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

6. Reagents, Methods & Materials

The reagent kit components can be used together with either of two CDs that only differ in the sample volume being analyzed. This will lead to different working ranges of the IgG titer assay. The user must determine which working range of the assay is required and order the appropriate kit.

- Gyrolab huIgG Kit - Low titer, including Bioaffy 1000 HC (working range: 1 to 20 000 ng/mL)
- Gyrolab huIgG Kit - High titer, including Bioaffy 20 HC (working range: 300 to 1 000 000 ng/mL)

Content	Product number
Gyrolab huIgG 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´)$_2$ 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 Bioaffy CD: One (1) Bioaffy 1000 HC or Bioaffy 20 HC	
Wash Station Solution 2: Gyrolab Wash Buffer pH 11	P0020087
96-well plate: Three 0.2 mL skirted PCR plates	P0004861
Microplate Foil: Three Microplate Foils	P0003313

Optional product

Intact **IgG**. Standard is provided as separate product, 50 µL at 4 mg/mL The standard needs to be ordered separately from Gyros Protein Technologies AB.	P0020379

Other Materials and Components required but not provided

- Gyrolab System
- Gyrolab Evaluator (version 3.5 or later)
- PBS-T; Bioaffy Pump Liquid and Bioaffy Wash Station 1 Solution – see Gyrolab User Guide
- Pipettes or pipetting equipment with disposable polypropylene tips
- Disposable polypropylene test tubes

- Plate centrifuge
- Microcentrifuge
- Vortex mixer
- Distilled or deionized water
- Filtering equipment with 0.22 or 0.45 µm filters

Gyrolab Software Requirements and Method

Gyrolab huIgG Kit is compatible with Gyrolab Control software versions 5.4 and higher.

The Gyrolab methods for Bioaffy 20 HC or Bioaffy 1000 HC, must be installed on the instrument being used for analysis. The methods can be downloaded from the Gyrolab User Zone at www.gyrosproteintechnologies.com/gyrolab-user-zone.

Please observe that for Gyrolab systems with software versions lower than 8.0, the method requires the use of three microtiter plates. These should be loaded with:

- Reagents
- Standard, controls and samples (one or several plates depending on number of samples)
- Wash buffers

respectively

7. Preparation of reagents

Note! Briefly spin all vials in a micro-centrifuge before opening to collect all liquid at the bottom.
Note! Ensure that pipetting routines are designed to minimize contamination, e.g. use clean pipettes and new tips. Contamination may reduce the utility/shelf life of the reagent.

Capture Reagent

The Capture Reagent (**Reagent A**) is ready-made and is transferred directly to the microtiter plate according to the Gyrolab Control Loading List.

Detection Reagent

The Detection Reagent (**Reagent B**) is ready-made and is transferred directly to the microtiter plate according to the Gyrolab Control Loading List.

Standard curve

The Gyrolab huIgG Kit does not include a standard. There are two options for preparing standards and QC controls:

- Select a representative IgG molecule at a known concentration. Standards and QC samples are diluted in Sample Dilution Buffer (provided in the Gyrolab IgG Titer Kit) according to the recommendations in Table 1 and 2.
- Order a standard from Gyros Protein Technologies (P0020379). This standard contains a human monoclonal IgG1 at 4 mg/mL in 50 µL. Standards and QC samples are diluted in Sample Dilution Buffer (provided in the Gyrolab IgG Titer Kit) according to the recommendations in Table 1 and 2.

Table 1 and 2 show examples of how to prepare standard curves for huIgG Kit High and Low titer, respectively, assuming the stock solution of IgG is 4 mg/mL. Select the CD depending on the desired working range of the assay and prepare the standard in accordance with recommendations in Table 1 and Table 2. Dilute the standard preparation in Sample Dilution Buffer (**Reagent E**).

Table 1 *Example of a standard curve for Gyrolab huIgG Kit - High titer intended to be used with Bioaffy 20 HC.*

High titer Dilution series (20 HC)	Concentration (ng/mL)	Volume stock (µL)	Volume higher concentration (µL)	Volume Reagent E (µL)
Stock	4 000 000			
Std 1	2 000 000	25		25
Std 2	400 000		10	40
Std 3	80 000		10	40
Std 4	16 000		10	40
Std 5	3 200		10	40
Std 6	640		10	40
Std 7	128		10	40
Blank	0		0	40

Table 2 *Example of a standard curve for Gyrolab huIgG Kit - Low titer intended to be used with Bioaffy 1000 HC.*

Low titer Dilution series (1000 HC)	Concentration (ng/mL)	Volume stock (µL)	Volume higher concentration (µL)	Volume Reagent E (µL)
Stock	4 000 000			
	500 000	10		70
	125 000		10	30
Std 1	25 000		10	40
Std 2	5 000		10	40
Std 3	500		10	90
Std 4	50		10	90
Std 5	10		10	40
Std 6	2		10	40
Std 7	0.5		10	30
Blank	Blank		0	40

QC samples

Table 3 shows an example of how to prepare QC samples from a stock solution of human monoclonal IgG at 4 mg/mL. Please note that different concentrations of QC samples are required for the high titer (Bioaffy 20 HC) and low titer (Bioaffy 1000 HC) versions of the assay. Table 3 gives guidance on the preparation of suitable concentrations.

Table 3. *Dilution of QC samples for different working ranges and IgG concentrations assuming a stock concentration of IgG at 4 mg/ml*

CD type	QC Control	IgG concentration (ng/mL)	Volume IgG stock (µL)	Volume higher QC concentration (µL)	Volume Reagent E (µL)
Bioaffy 20 HC	QC1 High	200 000	5		95
	QC2 High	10 000		5	95
	QC3 High	500		5	95
Bioaffy 1000 HC	QC1 Low	10 000		↓	↓
	QC2 Low	500			
	QC3 Low	25		5	95

8. Sample preparation

All samples to be analyzed for IgG titer are recommended to be centrifuged at 12 000 g for 4 min to sediment any aggregates or particulates that may be present. Transfer the sample into a new tube. Aspirate the sample with caution to avoid any sediment and transfer to a new tube. Dilute the sample in Reagent E. If the centrifugation step is omitted, extra caution might be required when evaluating the data, due to the risk of particulates causing spikes in the column profiles or clogged microstructures.

The working range of the IgG titer depends on which CD is used. Using Bioaffy 20 HC the working range is approximately 300 to 1 000 000 ng/mL. Using Bioaffy 1000 HC the working range is approximately 1 to 20 000 ng/mL

All samples must be diluted in Reagent E at least 1+1 by volume. However, depending on expected IgG titers, samples may have to be diluted further in Reagent E to hit the working range of the particular version of IgG titer assay selected.

9. Preparation of plate for wash buffers

Wash buffers (reagents C and D) shall be placed in a separate microtiter plate (100 µL in each well). For example, a full CD run will require three wells of Reagent C and two wells of Reagent D. The exact numbers are specified when the reagent plate is loaded.

10. Preparation of Wash Station Solution 2

Use freshly prepared solution

1. Dissolve one package (10 g) of Gyrolab Wash Buffer pH 11 powder in 1 L of deionized or distilled water.
2. Filter the solution through a 0.22 µm or 0.45 µm filter.

11. Data analysis

The data is evaluated in Gyrolab Evaluator version 3.3 or later. Open the run and click “Quantification”. In “Analysis Setup” default settings are recommended:

- do not include blanks in curve fitting
- five parameter logistic curve
- weight on response
- limit of detection factor: 2.

12. Performance characteristics

The performance of the Gyrolab huIgG Kit for the High and Low titer versions is illustrated in Fig.1. The combined analytical range covers approximately six orders of magnitude, making the kit applicable for many analytical situations in development of bioprocesses for monoclonal therapeutic antibodies.

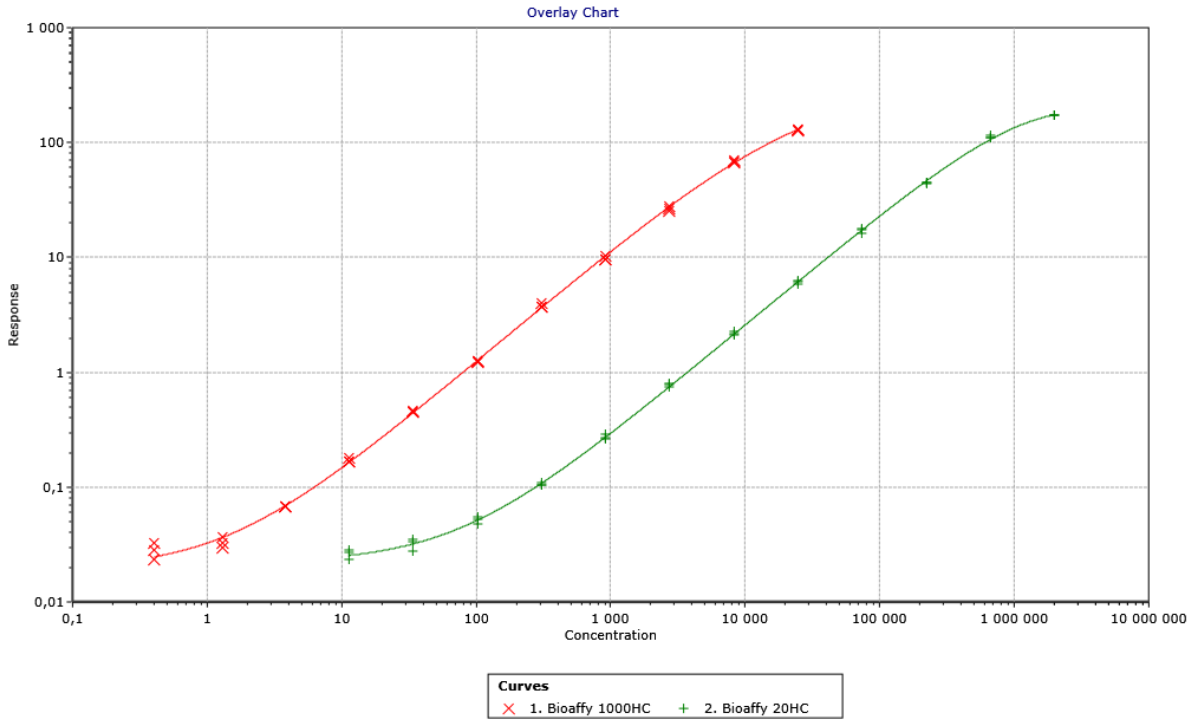

Fig.1. Working ranges for the Gyrolab huIgG Kit applied in Bioaffy 20 HC and Bioaffy 1000 HC, respectively. Concentrations are expressed in ng/mL.

12.1. Precision and Accuracy

Gyrolab huIgG Kit was evaluated by preparing standard curves ranging from ng/mL to µg/mL. Three runs of fresh standard curves were tested in triplicate in Bioaffy 1000 HC and Bioaffy 20 HC, respectively. The back-calculated intra- and inter-assay precision data are illustrated in Table 4 and 5. Table 6 and 7 show the back-calculated accuracy of standard points and QC samples for the High and Low titer kits, respectively.

***Table 4**. Intra and inter assay precision of Gyrolab huIgG Kit - High titer using Bioaffy 20 HC.*

	Gyrolab huIgG Kit – High titer				
Exp Conc. (ng/mL)		**Intra CD %CV**			**Inter CD / Run %CV (n=3)**
	n	**Run 1**	**Run 2**	**Run 3**	
11.3	3	73	28	70	56
33.9	3	13	7.7	30	22
102	3	26	11	1.2	17
305	3	11	11	14	10
914	3	5,8	7.8	5.5	6.9
2 743	3	12	5.9	3.8	6.3
8 230	3	2,5	4.6	2.3	3.0
24 691	3	1.9	0.91	2.8	2.2
74 074	3	1.5	0.24	3.1	4.1
222 222	3	0.63	3.0	2.6	2.1
666 667	3	3.2	4.9	6.1	6.3
2 000 000	3	5.3	6.0	3.7	4.5

***Table 5**. Intra and inter assay precision of Gyrolab huIgG Kit - Low titer using Bioaffy 1000 HC.*

	Gyrolab huIgG Kit – Low titer				
Exp Conc. (ng/mL)		**Intra CD %CV**			**Inter CD / Run %CV (n=3)**
	n	**Run 1**	**Run 2**	**Run 3**	
0.4	3	17	21	18	16
1.3	3	13	9.2	15	12
3.8	3	0.88	7.5	2.3	4.0
11.3	3	1.9	1.6	2.4	1.9
33.9	3	3.4	1.4	2.7	2.5
102	3	2.3	2.1	1.8	2.1
305	3	2.0	3.6	2.3	2.4
914	3	0.81	2.3	3.3	2.3
2743	3	5.4	0.84	0.79	2.9
8230	3	5.8	4.6	4.5	4.4
24691	3	6.2	10.5	8.8	7.4

Table 6. *Accuracy of Gyrolab huIgG Kit - High titer, based on back-calculated standards and separate QC controls.*

	Accuracy Gyrolab huIgG Kit – High titer							
			Run 1		**Run 2**		**Run 3**	
Identity	**n**	**Exp Conc. (ng/mL)**	**Av Conc. (ng/mL)**	**Av Bias %**	**Av Conc. (ng/mL)**	**Av Bias %**	**Av Conc. (ng/mL)**	**Av Bias %**
Std 1	3	11	16	41	13	14	12	3.6
Std 2	3	34	35	4.5	28	-17	40	19
Std 3	3	102	93	-8.4	115	13	104	2.2
Std 4	3	305	323	5.9	324	6.1	301	-1.3
Std 5	3	914	1 034	13	960	5.0	951	4.1
Std 6	3	2 743	2 683	-2.2	2 614	-4.7	2 773	1.1
Std 7	3	8 230	8 007	-2.7	7 826	-4.9	7 886	-4.2
Std 8	3	24 691	24 052	-2.6	23 851	-3.4	24 553	-0.56
Std 9	3	74 074	72 541	-2.1	77 817	5.1	71 654	-3.3
Std 10	3	222 222	220 828	-0.63	222 891	0.30	223 196	0.44
Std 11	3	666 667	727 343	9.1	740 225	11	811 780	22
Std 12	3	2 000 000	1 883 962	-5.8	1 842 184	-7.9	1 849 844	-7.5
QC 1 IgG1-a	3	300	291	-3.1	316	5.3	275	-8.3
QC 2 IgG2	3	300	289	-3.5	293	-2.4	325	8.3
QC 3 IgG4	3	300	293	-2.3	274	-8.6	266	-11
QC 4 IgG1-b	3	300	278	-7.3	302	0.52	286	-4.6

Table 7. *Accuracy of Gyrolab huIgG Kit - Low titer, based on back-calculated standards and separate QC controls.*

	Accuracy Gyrolab huIgG – Low titer							
			Run 1		**Run 2**		**Run 3**	
Identity	**n**	**Exp Conc. (ng/mL)**	**Ave Conc. (ng/mL)**	**Ave Bias %**	**Ave Conc. (ng/mL)**	**Ave Bias %**	**Ave Conc. (ng/mL)**	**Ave Bias %**
Std 1	**3**	**0.4**	0.47	18	0.43	7.8	0.44	9.6
Std 2	**3**	**1.3**	1.2	-10	1.3	-2.5	1.3	-3.1
Std 3	**3**	**3.8**	3.8	-0.94	3.7	-1.8	3.7	-2.7
Std 4	**3**	**11**	11	0.38	11	-1.6	11	-0.60
Std 5	**3**	**34**	36	5.2	35	4.3	35	2.7
Std 6	**3**	**102**	106	3.5	104	2.0	107	4.6
Std 7	**3**	**305**	307	0.59	309	1.3	311	1.8
Std 8	**3**	**914**	878	-3.9	898	-1.8	884	-3.3
Std 9	**3**	**2 743**	2 664	-2.9	2 639	-3.8	2 614	-4.7
Std 10	**3**	**8 230**	8 591	4.4	8 586	4.3	8 771	6.6
Std 11	**3**	**24 691**	24 620	-0.29	24 661	-0.12	24 461	-0.93
QC 1 IgG1-a	**3**	**10**	11	6.2	9.1	-9.1	8.4	-16
QC 2 IgG2	**3**	**10**	8.3	-17	8.4	-16	8.7	-13
QC 3 IgG4	**3**	**10**	7.6	-24	8.1	-19	8.1	-19
QC 4 IgG1-b	**3**	**10**	9.0	-9.5	11	5.4	10	-2.6

12.2. LOD, LLOQ, ULOQ of Gyrolab IgG titer Kit

Limit of Detection (LOD) was determined from measurements of standard points and blanks in three runs (nine data points using Gyrolab IgG titer Kit).

Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) were determined by analyzing several QC samples in the lower and upper range of the assay, respectively. The lowest (LLOQ) and highest (ULOQ) huIgG concentration that gave a Total Error (% Relative Error + %Coefficient of Variation) < 30 % were assigned as LLOQ and ULOQ, respectively, for the two Bioaffy CDs. The working range of the Gyrolab huIgG Kit assay is summarized in Table 8.

Table 8. *Assay characteristics of Gyrolab huIgG Kit.*

Assay Characteristics (ng/mL)		
	Gyrolab huIgG Kit – High titer	**Gyrolab huIgG Kit – Low titer**
LOD	100	0.5
LLOQ	300	1
ULOQ	1 000 000	20 000

12.3. Assay compatibility with different human IgG subclasses

Therapeutic antibodies (pharmaceutical grade) of different subclasses were evaluated for dilutional linearity in Gyrolab IgG titer assays using Bioaffy 20 HC and Bioaffy 1000 HC. Highly concentrated antibody preparations were serially diluted in Reagent E to cover ranges of 10^4 (Bioaffy 1000 HC) and 10^5 (Bioaffy 20 HC). See Table 9 for the results.

Table 9*. Range of linear dilution determined on back-calculated concentrations of serially diluted pharmaceutical grade therapeutic antibodies of different IgG subclasses.*

	Ranges of linear dilution exceeding 80 % of the highest back-calculated concentration of individual IgG subclasses		
IgG subclass	**Human IgG1**	**Human IgG2**	**Human IgG4**
Gyrolab huIgG Kit – High titer	3125	625	3125
Gyrolab huIgG Kit – Low titer	625	125	625

13. Troubleshooting

Please visit the kit guidelines section on www.gyrosproteintechnologies.com/gyrolab-user-zone for more information and tips or contact your local field application specialist for support.

14. Supplementary Reagents

Description	**Catalogue number**	**Amount**
Gyrolab Wash Buffer pH 11	P0020096	10 pc
PCR plates 96	P0004861	50 pc
Microplate Foils	P0003313	50 pc

Disposal procedures

Gyrolab CDs and microplates should be disposed of in accordance with federal, state and local environmental control regulations. The user is responsible for waste disposal and for providing suitable waste containers. Packaging material can be disposed of through combustion for energy recovery.