Instruction For Use

Gyrolab® Generic TK Kit

Product number	Product Name
P0020500	Gyrolab Generic TK Kit
P0020806	Gyrolab Generic TK CD50 Type E Kit
P0020808	Gyrolab Generic TK CD50 Type F Kit

1. Product description

Gyrolab® Generic TK kit is a ready-to-use kit used for toxicokinetic (TK) analysis on Gyrolab® systems of intact human IgG (IgG1, IgG2, IgG4) in non-human serum matrices. The ready-made reagents are provided in a kit format to be used together with a single Bioaffy 20 HC CD. Gyrolab Generic TK kit does not include standard.

P0020500 Gyrolab Generic TK Kit is a single CD kit containing all reagents, CDs, buffers and consumables required for 112 datapoints.

P0020806 Gyrolab Generic TK CD50 type E Kit and P0020808 Gyrolab Generic TK CD50 type F Kit are manufactured on order and contain all reagents, CDs, buffers and consumables required for 50 CD runs (50*112 data points). The reagents are packed in 10 plastic bags with each bag containing reagent volumes in vials that are sufficient for 5 single CD runs. Please assess which buffer your samples require using P0020500 Gyrolab Generic PK Kit before ordering the CD50 kit.

2. Intended use

This kit is intended for quantification of intact human IgG (IgG1, IgG2, IgG4) in non-human serum matrices, using the Gyrolab immunoassay instrument and the Gyrolab Bioaffy™ 20 HC CD and ready-to-use reagents.

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

3. Introduction

Toxicity studies include evaluation of toxicological effects from systemic exposure to a recombinant therapeutic IgG molecule under development. Toxicokinetic (TK) monitoring is performed in pre-clinical animal models to support toxicity studies for assessment of safety prior to initiation of clinical studies.

Gyrolab Generic TK Kit reagents and protocols have been optimized for the determination of human intact IgG in cynomolgus monkey, mouse, rat, dog and rabbit, thereby reducing assay development and the number of assays.

4. Assay principle

Gyros Protein Technologies has developed a generic kit for TK analysis that can quantify intact human IgG (IgG1, IgG2, and IgG4) in non-human serum matrices, such as from cynomolgus monkey, mouse, rat, dog, and rabbit. The kit is based on a set of reagents that are used in combination with a single Gyrolab Bioaffy 20 HC CD.

A capturing molecule, biotinylated anti-human IgG-Fc, is introduced into a microstructure in the CD to saturate a capture column packed with porous beads coupled with streptavidin. Samples are introduced into the microstructures, volume-defined and captured in the capture column. The detection reagent, anti-human IgG antibody labeled with Alexa Fluor® 647, is then added and the fluorescence signal is measured. The integrated signal in the capture column represents the total response from the sample.

5. Limitations

Partial Use of Kits
We recommend no more than two times partial use of reagent kit and CD that have been removed from its pouch. The reagents and CD should be used within one week.

Assay Qualification
Gyrolab Generic TK Kit is intended for the quantification of intact human monoclonal IgG in non-human serum matrices. Gyrolab Generic TK Kit is compatible with monoclonal human 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, precision, and minimal required dilution (MRD).

6. Storage and Stability

Reagents
Gyrolab Generic TK 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.

Opened CD package
CDs must be used within one week of opening. Return partially used CDs to original CD pouch. Re-seal. Store dark, dry and at ambient temperature.

Opened Capture and Detection Reagent
Capture and Detection Reagents that has been rethawed and partially used can be stored up to one week in +4 to +8°C.

7. Reagents, Methods & Materials

Contents	Product Number

P0020500 Gyrolab Generic TK Kit contents

This product contains 1 CD and all reagents required for 1 CD run (112 data points).

Gyrolab Generic TK Kit Reagents:

Reagent A: Capture Reagent, biotinylated anti-human IgG-Fc[1]. Ready to use solution, 75 µL
Reagent B: Detection Reagent, Alexa Fluor® 647 labeled anti-human IgG. Ready to use solution, 75 µL
Reagent C: Wash Buffer 1, 1 mL
Reagent D: Wash Buffer 2, 1 mL
Reagent E: Sample Dilution Buffer, 25 mL
Reagent F: Alternative Sample Dilution buffer (primarily for molecules with pI>8), 25 mL

Gyrolab Bioaffy CD: Bioaffy 20 HC	**P0004424**
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**

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GYROS PROTEIN Technologies

P0020806 Gyrolab Generic TK CD50 Type E Kit and P0020808 Gyrolab Generic TK CD50 Type F Kit contents

This product contains all reagents, CDs and consumables required for 50 CD runs (50x112 data points). All reagents and CDs are from the same manufacturing lot. The reagents are packed in 10 plastic bags with reagents enough for 5 CDs. Each CD50 kit contains the following:

Gyrolab Generic TK CD50 Type E and F Kit Reagents:

Reagent A: Capture Reagent, biotinylated anti-human IgG-Fc[1]. Ready to use solution, 275 µL

Reagent B: Detection Reagent, Alexa Fluor® 647 labeled anti-human IgG. Ready to use solution, 275 µL

Reagent C: Wash Buffer 1, 2 mL

Reagent D: Wash Buffer 2, 2 mL

Reagent E: Sample Dilution Buffer, 2 x 25 mL (P0020806 Generic TK CD50 Type E) or **Reagent F**: Two (2) Alternative Sample Dilution buffer (primarily for molecules with pI>8), 2 x 25 mL (P0020808 Generic TK CD50 Type F)

Gyrolab Bioaffy CD: Fifty (50) Bioaffy 20 HC	**P0004424**
Wash Station Solution 2: Fifty (50) vials of Gyrolab Wash Buffer pH 11	**P0020087**
96-well plate: One hundred and fifty (150) 0.2 mL skirted PCR plates	**P0004861**
Microplate Foil: One hundred and fifty (150) Microplate Foils	**P0003313**

Other Materials and Components required but not provided:

- Gyrolab System (Control Software version 5.4 or later)
- Gyrolab Evaluator (version 3.3 or later)
- PBS-T; Bioaffy Pump Liquid and Bioaffy Wash Station Solution – see Gyrolab User Guide
- Pipettes or pipetting equipment with disposable polypropylene tips
- Disposable polypropylene test tubes
- Lab centrifuge
- Vortex mixer
- Filtering equipment with 0.22 or 0.45 µm filters

[1]Made with Thermo Scientific™ CaptureSelect™ Human IgG-Fc TK Biotin Conjugate from Thermo Fisher Scientific Inc. and its subsidiaries. Thermo Scientific and CaptureSelect are trademarks of Thermo Fisher Scientific Inc. and its subsidiaries.

Optional product

Standard is not included in the kit but can be ordered separately

Intact recombinant human monoclonal IgG1, 50 µL at 4 mg/mL	P0020379

Note that Gyros Protein Technologies recommends the drug molecule of interest should be used as reference standard.

CD design

For instruments with Gyrolab Control Software versions older than 7.1.2, a new CD design, “Gyrolab Bioaffy 20 HC CD”, must be imported prior to the first analysis. The CD design can be downloaded from Gyrolab User Zone at www.gyrosproteintechnologies.com/gyrolab-user-zone.

Gyrolab Software Requirements and Method

The Gyrolab method, **Generic TK kit method**, must be installed on the instrument being used for analysis. If not already installed in the database, the method can be downloaded from the Gyrolab User Zone at www.gyrosproteintechnologies.com/gyrolab-user-zone.

For information on how to import a method and CD design, please refer to Gyrolab User Guide or contact Gyros Protein Technologies.

8. Preparations

- Equilibrate the CD at room temperature for at least 30 minutes in the unopened aluminum pouch.
- Prepare PBS-T.
- Prepare Wash Station solution 2.
- Dissolve one package (10 g) of Gyrolab Wash Buffer pH 11 powder in 1 L of deionized or distilled water for a one-CD run or dissolve two packages (20 g) of Gyrolab Wash Buffer pH 11 powder in 2 L of deionized or distilled water for a five-CD run.
- Filter the solution through a 0.22 or 0.45 µm filter. Prepare fresh wash solution weekly. **Note! The needle wash procedures in the Generic TK kit method have been carefully optimized to minimize carry-over from high concentrations of IgG. The consumption of wash buffer solutions 1 and 2 used by the method are therefore higher than other Gyrolab methods.**

Required volume

- 1000 mL for 1 CD
- 2000 mL for 2-3 CDs
- 3000 mL for 4-5 CDs

- Prepare a standard curve and quality control samples (QC) as suggested in section 9.
- Prepare the samples according to section 10.
- Capture and detection reagents are ready-to-use and transferred directly to the microtiter plate according to the Gyrolab Loading List. Allow the capture (**Reagent A)** and detection (**Reagent B**) reagents to reach room temperature before transferring to the microtiter plate. **Note! Briefly spin all vials in a micro-centrifuge before opening to collect all liquid at the bottom.**
- Place reagents, standards, controls, samples and wash buffers to provided 96-well plate according to section 11.

9. Standard curve and Quality Control samples

We recommend that you select a representative IgG molecule at a known concentration, preferable the same IgG molecule to be analyzed, for standards and QC samples. Table 1 shows examples of how to prepare standard curves for Gyrolab Generic TK Kit. Prepare the standard in accordance with recommendations for Gyrolab Generic TK Kit. Dilute the standard preparation in the same matrix concentration as the samples – see Section 13 on how to determine Minimum Required Dilution (MRD) and select sample dilution buffer.

Table 1. *Example of a standard curve for Gyrolab Generic TK Kit using Bioaffy 20 HC*

Dilution series (20 HC)	Concentration (µg/mL)	Volume higher concentration (µL)	Volume Matrix (Serum in Reagent E or Reagent F) (µL)
Stock	xx		
Std 1	800	x	xx
Std 2	200	15	5
Std 3	50	15	45
Std 4	12.5	15	45
Std 5	3.125	15	45
Std 6	0.781	15	45
Std 7	0.195	15	45
Blank	Blank	0	45

QC samples

Table 2 shows an example of how to prepare QC samples from a stock solution of human monoclonal IgG.

Table 2. *Example of dilutions of QC samples*

QC Control	IgG concentration (µg/mL)	Volume higher QC concentration (µL)	Volume matrix (serum in Reagent E or Reagent F) (µL)
Pre 1	1 000	x	x
QC1 High	250	10 µl of Pre 1	30
QC2 Medium	25	10	90
QC3 Low	3	12	88

10. Sample preparation

All samples to be analyzed should be centrifuged at 12 000 g for 4 min to sediment any particulates. Aspirate the sample with caution to avoid any sediment and transfer to a new tube. Dilute the sample in Reagent E or Reagent F – see section 13 for buffer selection and how to determine minimum required dilution, typically ≤50%. If the centrifugation step is omitted, extra caution might be required when evaluating the data, as spikes in the column profiles or possibly also clogged microstructures could occur.

11. Placement of reagents, controls, samples and wash buffer in 96-well plate/plates for Gyrolab run

For Gyrolab Control Software v 8.0 and above
Place reagents, standards, controls, samples and Wash Buffers in the 96-well microplate according to the loading list. Seal the plates with microplate foil and briefly spin the microplates in a plate centrifuge to collect all liquid at the bottom. Examine microplates after centrifugation to make sure that no bubbles are present.

For Gyrolab Control Software versions below 8.0
Wash Buffers shall be placed in a separate 96-well plate provided (100 µL in each well) in wells selected by the user. As an example, a full CD run will require three wells of Wash Buffer 1 (reagent C) and two wells of Wash Buffer 2 (reagent D). The exact numbers are specified when the reagent plate is loaded.

12. 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

13. Assay development

The first time a new molecule is analyzed, or the species is changed we recommend that you determine minimum required dilution, MRD, and optimal sample dilution buffer. Two sample dilution buffers are provided in Gyrolab Generic TK Kit: one for molecules with a pI (isoelectric point) around and below 8.5 (**Reagent E**) and one for molecules with a pI above 8.5 (**Reagent F**). Note, that these pI intervals are only a guideline for determining the buffer to use for a certain molecule. When determining the optimal sample dilution buffer, we recommend that you run a 12-point standard curve with the molecule and matrix. An MRD of 50% is a good starting point. An example of a standard curve and how to analyze the result is shown in appendix A. Thereafter, determine the MRD for the molecule to be tested in the selected matrix and with the selected buffer.

14. Performance characteristics

The performance of Gyrolab Generic TK Kit for different species (IgG1) is illustrated in **Figure 1** and the specificity for the kit is illustrated in **Figure 2**.

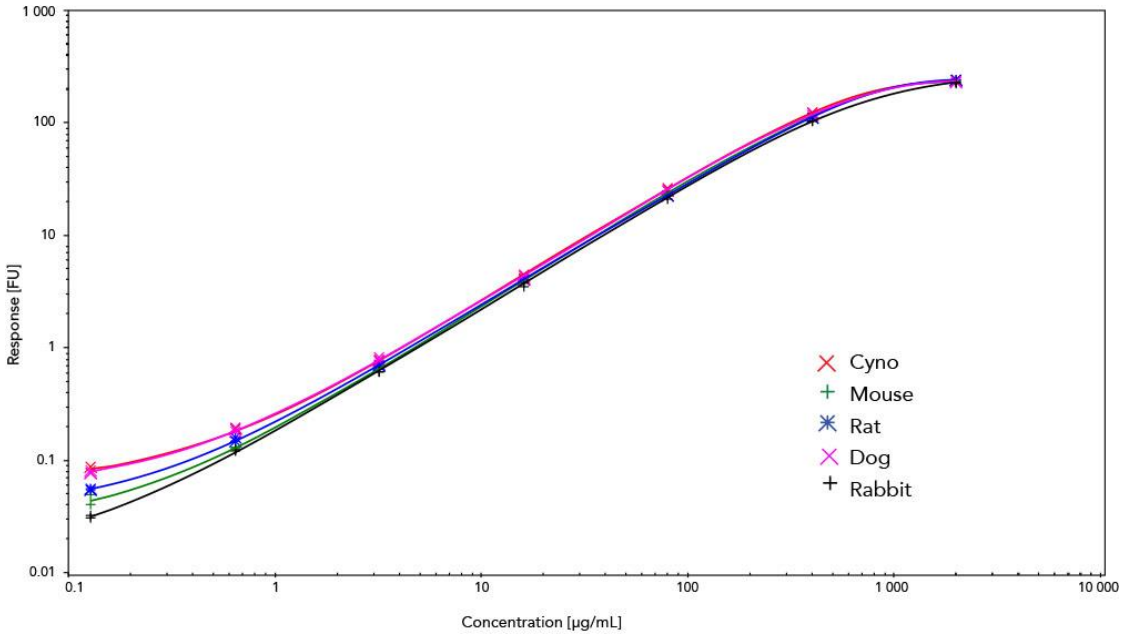

Figure 1. *Working ranges for different species in 50% serum using Gyrolab Generic TK Kit. Concentrations are expressed in µg/mL.*

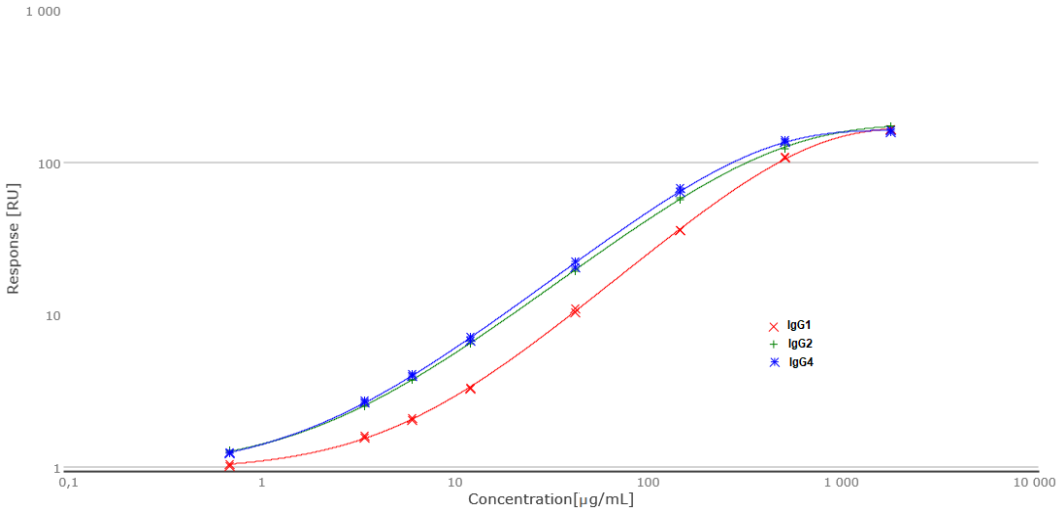

Figure 2. *Specificity for Gyrolab Generic TK Kit for IgG1, IgG2, IgG4 in 50% cyno serum. Concentrations are expressed in µg/mL*

15. 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.

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.

Appendix A

Buffer selection experiment

To select the optimal sample dilution buffer we suggest that you run a 12-point standard curve (see Table 3 below for an example of the standard curve to be used) with the molecule and matrix to be analyzed using the TK assay. Start with Reagent E, run the experiment and analyze the result. See Figure and Figure for examples. If the coefficient of variation, CV, is high in the lower end of the standard curve then run an additional experiment with Reagent F.

Table 3. *Example of a 12-point standard curve for buffer selection on Bioaffy 20 HC using Gyrolab Generic TK Kit.*

High titer Dilution series (20 HC)	Concentration (µg/mL)	Volume higher concentration (µL)	Volume matrix (serum in Reagent E or F) (µL)
Std 1	1 000	x	x
Std 2	333	10	20
Std 3	111	10	20
Std 4	37	10	20
Std 5	12.3	10	20
Std 6	4.1	10	20
Std 7	1.3	10	20
Std 8	0.46	10	20
Std 9	0.15	10	20
Std 10	0.05	10	20
Std 11	0.017	10	20
Blank	0	0	20

Analyze the results in Gyrolab Evaluator. See below for examples of results using non-optimal and optimal buffers.

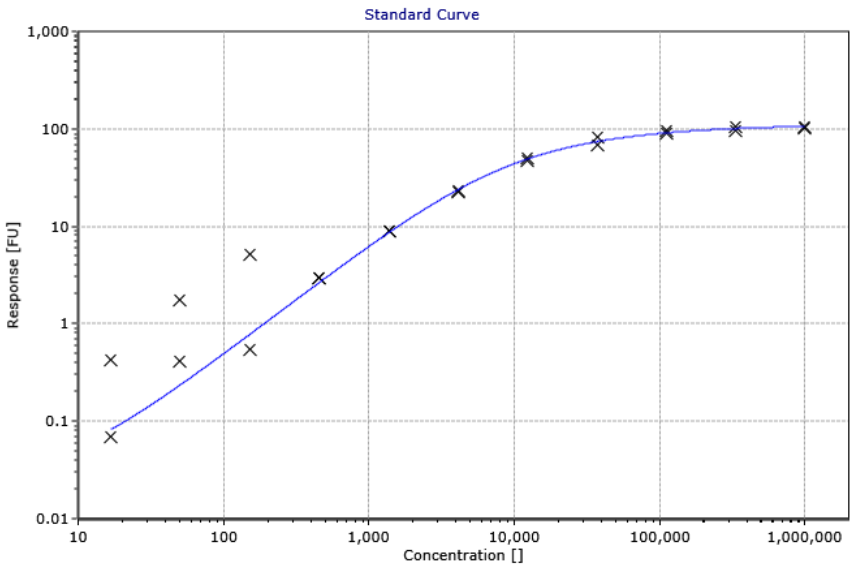

Figure 3. *Example of a 12-point standard curve in 50% Cyno serum and a buffer that is not optimal for the molecule to be analyzed. Concentrations are expressed in ng/mL*

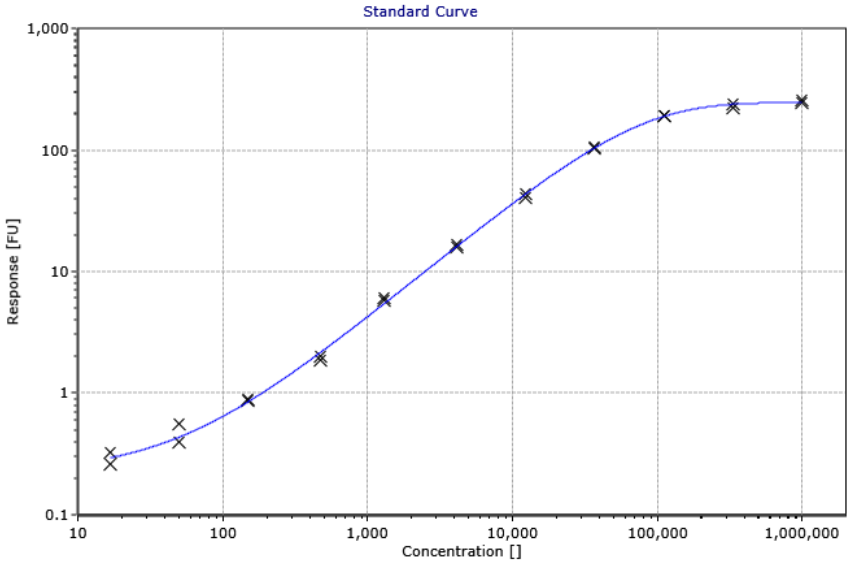

Figure 4. *Example of a 12-point standard curve in 50% Cyno serum and a buffer that is optimal for the molecule to be analyzed. Concentrations are expressed in ng/mL*