Gyrolab® 2G Generic PK Kit Reagents

Product Information Sheet D0044852/C

- Short time to results – 80 minutes
  - Optimized kit reagents ensure assay robustness, convenience and consistency of results
  - Easy to use
  - No need for assay development
- Broad dynamic range and low sample consumption
  - High sensitivity and wide dynamic range of three logs
  - Minimizes dilutions and repeats
  - Enables serial analysis of samples from small animals
- Meets preclinical study needs
  - Different matrices: mouse, rat, cynomolgus monkey, rabbit, and dog
  - Range of IgG classes: IgG1, IgG2, and IgG4
  - Reactive against IgGs containing kappa (κ) light chain
  - Can be combined with different Gyroab® Bioaffy™ disc types for more flexibility

Introduction

The development of recombinant IgG therapeutic drugs is challenging and often involves several candidates per program. In addition, the efficacy and safety parameters of lead candidates must be evaluated in two preclinical species, such as mouse and cynomolgus (cyno) monkey. Assays to detect the biotherapeutic in biological fluids for these studies have challenges including limited sample volumes and a wide range of analyte levels.

Gyrolab® systems are widely used with great success in pharmacokinetic (PK) studies in preclinical and clinical development in many biopharmaceutical companies. To perform PK studies of human antibodies in non-human species, it is necessary to have an assay that selectively recognizes human IgG from the endogenous non-human IgG. To meet these needs, Gyros Protein Technologies has developed Gyrolab 2G Generic PK Kit Reagents to accurately and robustly quantify human IgG1, IgG2, or IgG4 levels over a broad working range in several matrices and with the convenience that ready-to-use kits brings. The Gyrolab 2G Generic PK Kit Reagents have been optimized to obtain the best assay performance in combination with the Gyrolab Bioaffy 1000 HC disc. Gyrolab assays consume only nanoliter volumes of sample, providing major benefits by enabling serial sampling of fewer mice to generate better data for more analyses while using less material.

Combined with the automation and throughput of Gyrolab systems, the kit reagents help to accelerate studies throughout early-stage and pre-clinical development of recombinant human intact antibodies of different IgG subclasses in a range of species. This reduces time to market and increases productivity and cost effectiveness while maintaining product quality requirements.

GYROS PROTEIN Technologies

The assay

Gyrolab 2G Generic PK Kit Reagents have been developed to quantify intact human IgG (IgG1, IgG2, IgG4) in sera from a range of species. The kit reagents add convenience and significant workflow benefits compared to ELISA and results are generated in 80 minutes. Combined with Gyrolab Bioaffy 1000 HC disc, the kit reagents cover a broad dynamic range of three to four logs to work with a wide range of sample types, and the automation provided by Gyrolab systems significantly reduces the number of manual steps and risk for error, as well as shortening the time to results to only 80 minutes per assay.

Gyrolab 2G Generic PK Kit reagents are optimized to be used with Gyrolab Bioaffy 1000 HC disc providing working ranges typical for PK studies. However, to answer even more needs, the kit reagents offer a versatile solution as they can be used with other Gyrolab Bioaffy disc types to adapt the dynamic range if required (note that in this case, assay optimization may be needed).

The sandwich assay (Figure 1) is based on the biotinylated anti-human IgG-Fc[1] as a capturing reagent and anti-human IgG labeled with Alexa Fluor™ 647 as a detection reagent. The biotinylated reagent is introduced into a microstructure in the Gyrolab Bioaffy microfluidic disc to saturate a capture column packed with beads that are coupled with streptavidin. Subsequently, diluted sera containing intact human IgG are volume-defined and introduced into the microstructures where intact IgG is captured on the affinity capture column. The fluorescently labeled detection antibody is added and samples are quantified using Gyrolab systems.

Gyrolab 2G Generic PK Kit Reagents do not contain a standard and it is strongly recommended that the molecule of interest is used as a reference standard.

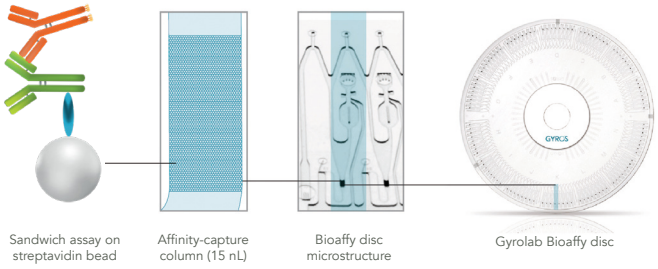

Figure 1. Sandwich immunoassay format.

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

Kit performance

Broad dynamic range

Figure 2 shows the assay ranges of standard curves for a human IgG1 monoclonal antibody in serum from different species (cyno, dog, mouse, rabbit and rat) using Gyrolab Bioaffy 1000 HC disc.

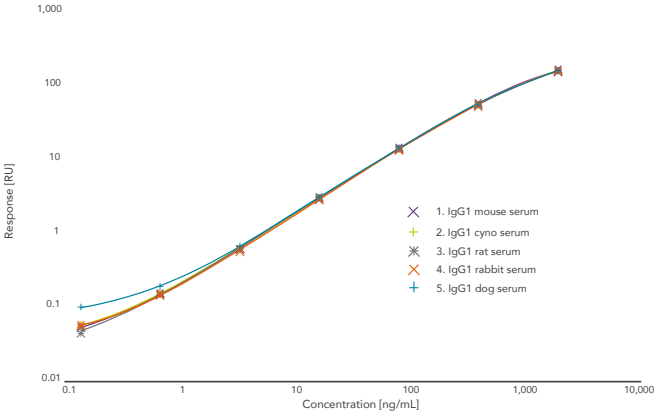

Figure 2. Comparison of standard curves prepared in 10% mouse, cyno, rat, rabbit and dog serum, with intact human IgG1 used as analyte.

Working with the kit reagents, an optimal Minimum Required Dilution (MRD) should be established. For PK, the MRD is typically 1:10 corresponding to 10% serum. MRD should be determined for each new molecule to be analyzed.

High precision and accuracy

The intra- and inter-assay data for 6 independent runs using a human IgG1 monoclonal antibody in 10% mouse serum (Table 1) and in 10% cynomolgus monkey serum (Table 2) demonstrate high accuracy and precision with low %CV and %RE in both species.

Table 1. Intra- and inter-assay precision and relative error using Gyrolab 2G Generic PK Kit Reagents with Gyrolab Bioaffy 1000 HC disc for a standard curve generated with a monoclonal antibody of IgG1 subtype in 10% mouse serum. QCs (in 10% matrix) were run as triplicate in 6 independent runs.

Sample	Conc. (ng/mL)	Intra run %CV (n=6)	Inter run %CV (n=6)	Average |%RE| (n=6)
QC1	1200	1.2 - 11.6	9.3	10.4
QC2	50	1.0 - 9.3	6.3	6.6
QC3	2	1.1 - 6.1	5.3	6.3

Table 2. Intra- and inter-assay precision and relative error using Gyrolab 2G Generic PK Kit Reagents with Gyrolab Bioaffy 1000 HC disc for a standard curve generated with a monoclonal antibody of IgG1 subtype in 10% cynomolgus monkey serum. QCs (in 10% matrix) were run as triplicate in 6 independent runs.

Sample	Conc. (ng/mL)	Intra run %CV (n=6)	Inter run %CV (n=6)	Average |%RE| (n=6)
QC1	1200	0.9 - 13.0	9.2	9.3
QC2	50	1.8 - 7.7	6.2	7.4
QC3	2	2.2 - 9.9	8.9	10.0

Good specificity for IgG1, IgG2 and IgG4

Kit reagents are compatible with human kappa (κ) light chain containing IgG1, IgG2 and IgG4 subtypes (Figure 3), which represent most of the clinically approved therapeutic antibodies.

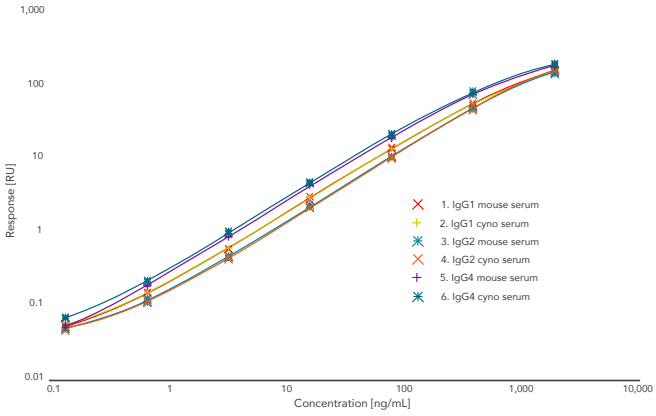

Figure 3. Specificity of human IgG1, IgG2, and IgG4 in 10% cyno and mouse serum using Gyrolab 2G Generic PK Kit Reagents with Gyrolab Bioaffy 1000 HC disc.

High dilutional linearity

Dilutional linearity was evaluated by spiking an intact human IgG1 molecule in both neat (100%) matrix and in 10% matrix, using two mouse (Table 3) and two cyno (Table 4) serum samples. All samples were run at MRD 1:10.

Except for one dilution with 126% recovery in mouse samples and one dilution with 78% recovery (at LLOQ level) in cyno samples, all samples and dilutions were within 80-120% recovery. These data demonstrate high linearity obtained from dilution 10x to 10 240x, covering the whole assay range on the Gyrolab Bioaffy 1000 HC disc.

Table 3. Recovery data of mouse serum samples spiked with an intact human IgG1 molecule in both 100% and 10% matrix (all samples run at 10% in assay). Dilution 10 corresponding an on-plate concentration of 1000 ng/mL and dilution 10240 corresponding an on-plate concentration of 0.98 ng/mL.

Dilution	Recovery (%)			
	Mouse serum sample 1		Mouse serum sample 2	
	Spiked in 100% serum	Spiked in 10% serum	Spiked in 100% serum	Spiked in 10% serum
10	106	106	104	112
40	104	98	103	94
160	109	98	112	100
640	105	94	119	101
2560	110	89	126	96
10240	91	103	118	94

Table 4. Recovery data of cyno serum samples spiked with an intact human IgG1 molecule in both 100% and 10% matrix (all samples run at 10% in assay). Dilution 10 corresponding an on-plate concentration of 1000 ng/mL and dilution 10240 corresponding an on-plate concentration of 0.98 ng/mL.

Dilution	Recovery (%)			
	Cyno serum sample 1		Cyno serum sample 2	
	Spiked in 100% serum	Spiked in 10% serum	Spiked in 100% serum	Spiked in 10% serum
10	104	96	113	110
40	84	88	92	96
160	86	91	92	94
640	86	96	87	94
2560	80	92	80	91
10240	80	83	78	86

Ordering Information

Product Number	Product name	Description
P0020969B	Gyrolab 2G Generic PK Type E Kit	Includes Kit Reagents, Gyrolab Bioaffy 1000 HC disc and accessories to generate 96 datapoints (1 disc). Note that standard is NOT included in Kit Reagents.
P0020970B	Gyrolab 2G Generic PK Type F Kit	Includes Kit Reagents, Gyrolab Bioaffy 1000 HC disc and accessories to generate 96 datapoints (1 disc). Primarily for molecules with pI > 8. Note that standard is NOT included in Kit Reagents.
P0020971B	Gyrolab 2G Generic PK CD50 Type E Kit	Includes Kit Reagents, Gyrolab Bioaffy 1000 HC discs and accessories to generate 4800 datapoints (50 discs). Note that standard is NOT included in Kit Reagents.
P0020972B	Gyrolab 2G Generic PK CD50 Type F Kit	Includes Kit Reagents, Gyrolab Bioaffy 1000 HC discs and accessories to generate 4800 datapoints (50 discs). Primarily for molecules with pI > 8. Note that standard is NOT included in Kit Reagents.
Components of Gyrolab 2G Generic PK Type E Kit (P0020969) or Type F Kit (P0020970) can be ordered separately		
P0020957 or P0020958	Gyrolab 2G Generic PK Type E Kit Reagents or Gyrolab 2G Generic PK Type F Kit Reagents	Includes Kit Reagents, sample dilution and wash buffers to generate 96 datapoints (1 disc). Type F is primarily for molecules with pI>8. Note that standard is NOT included in Kit Reagents.
P0020245	Gyrolab Bioaffy 1000 HC disc	1000 nL, 96 datapoints.
P0020863	Gyrolab Accessories Bundle	PCR Plate 96 (x3), Microplate foil (x3) and Gyrolab Wash station solution 2 (x1).

All products above can also be purchased separately as stand-alone products.

Gyrolab 2G Generic PK Type E (P0020957) or Type F (P0020958) Kit Reagents Contents

Reagent A Ready-to-Use Capture Reagent Biotinylated anti-human IgG-Fc

Reagent B Ready-to-Use Detection Reagent Alexa Fluor™ 647 labeled anti-human IgG

Reagent C Wash buffer 1

Reagent D Wash buffer 2

Reagent E or **F** Sample dilution buffer Type E or Type F (Primarily for molecules with pI > 8)

Note that standard is NOT included in kit reagents.

Storage conditions

Gyrolab Bioaffy 1000 HC disc

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

Shelf life (unopened package): see product label.

Gyrolab 2G Generic PK Type E or F 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:

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