Gyrolab® Generic PK/TK Kits

Product Information Sheet D0027811/F

- **Short time to results - 70 minutes**
  - Optimized kits ensure assay robustness, convenience and consistency of results
  - Easy to use
  - No need for assay development
- **Broad dynamic range and low sample consumption**
  - Dynamic range of three logs for each kit
  - Minimizes dilutions and repeats
  - Enables serial analysis of samples from small animals
- **Meets many needs**
  - Different matrices: mouse, rat, cynomolgus monkey, rabbit, and dog
  - Range of IgG classes: IgG1, IgG2, and IgG4

Introduction

The development of recombinant IgG therapeutic drugs is challenging and often involves several candidates per program in combination with reagent selection. In addition, the efficacy and safety parameters of lead candidates must be evaluated in two pre-clinical species, such as mouse, and cynomolgus monkey. Sample volumes may be limited and analyte levels may vary greatly.

Gyrolab® systems are widely used with great success in pharmacokinetic (PK) and toxicokinetic (TK) studies in preclinical and clinical development in many biopharmaceutical companies. To perform PK or TK 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 two kits to accurately and robustly quantify human IgG1, IgG2, or IgG4 titer over broad working ranges in several matrices and with the convenience that ready-to-use kits brings. The kits are based on the same reagents and are compatible with two Gyrolab Bioaffy CDs that differ in sample volume to cover concentration ranges which meet the needs for PK and TK studies. Moreover, 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 kits 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 Generic PK and TK Kits have been developed to quantify intact human IgG (IgG1, IgG2, IgG4) in sera from a range of species. The kits add convenience and significant workflow benefits compared to ELISA and results are generated in 70 minutes. Combined, the two kits cover a broad dynamic range of five 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 70 minutes per kit.

Gyrolab Generic PK and TK Kits are based on the same reagents and cover working ranges suitable for PK studies and TK studies respectively. Gyrolab Generic PK kit uses Bioaffy 1000 HC CD for working ranges typical for PK studies and Gyrolab Generic TK Kit is supplied with a Bioaffy 20 HC CD to work in the higher TK range.

The sandwich assay (Figure 1) is based on the biotinylated anti-human IgG-Fc1 as a capturing reagent and anti-human IgG antibody labeled with Alexa Fluor® 647 as a detection reagent. The biotinylated reagent is introduced into a microstructure in the Gyrolab Bioaffy CD to saturate a capture column packed with porous 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 Generic PK and TK Kits do not contain a standard and it is strongly recommended that the molecule of interest is used as a reference standard.

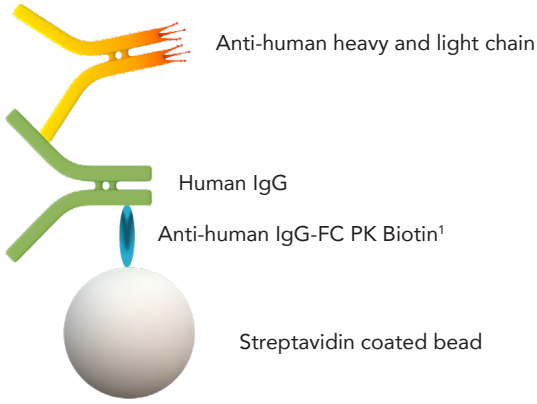

Figure 1. Sandwich assay format

[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 (cynomolgus monkey, dog, mouse, rabbit and rat) using both kits.

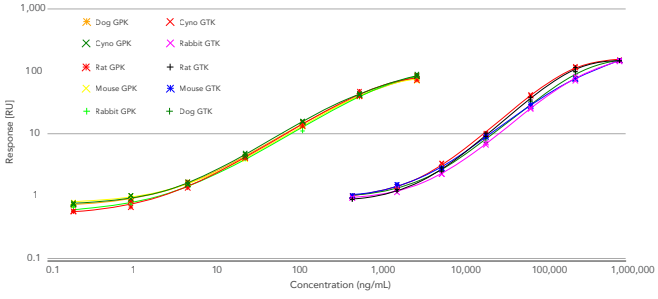

Figure 2. Gyrolab Generic PK and TK Kits cover a broad working range. This example shows results from both kits for a human IgG1 monoclonal antibody in neat serum in different species (cynomolgus monkey, dog, mouse, rabbit and rat).

Working with the kits an optimal MRD should be established. For PK, the MRD is typically 1:10 corresponding to 10% serum, while for TK the MRD is typically 1:2 corresponding to 50% serum. MRD should be determined for each new molecule to be analyzed.

High precision and accuracy

The data for five standard curves for each kit using a human IgG1 monoclonal antibody in 10% (PK Kit) and 50% (TK Kit) cynomolgus monkey serum demonstrate linearity over a broad dynamic range with low intra- and inter-assay variation (Figure 3). Table 1 shows typical inter- and intra-assay precision data using Gyrolab Generic PK Kit for a monoclonal antibody of IgG1 subtype

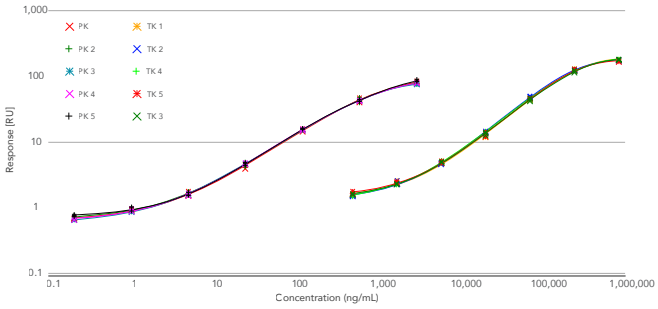

Figure 3. Overlay plots of five standard curves for each kit over their respective assay range using a human IgG1 monoclonal antibody in 10% serum (PK Kit) and 50% serum (TK Kit).

Table 1. Inter- and intra-assay precision using Gyrolab Generic PK Kit for a standard curve generated with a monoclonal antibody of IgG1 subtype.

	Conc. (ng/mL)	Avg. response	Intra run %CV (n=5)	Inter run %CV (n= 5)	%RE	%TE*
QC 1	2000	74.0	10.3	2.0	-7.6	18
QC 2	50	6.9	6.7	3.0	10.2	18
QC 3	4	1.4	7.3	2.4	9.9	18

* %TE = %CV+|%RE|

Good specificity for IgG1, IgG2 and IgG4

Both kits are compatible with human IgG1, IgG2 and IgG4 subtypes (figure 4), which represent most clinically approved therapeutic antibodies.

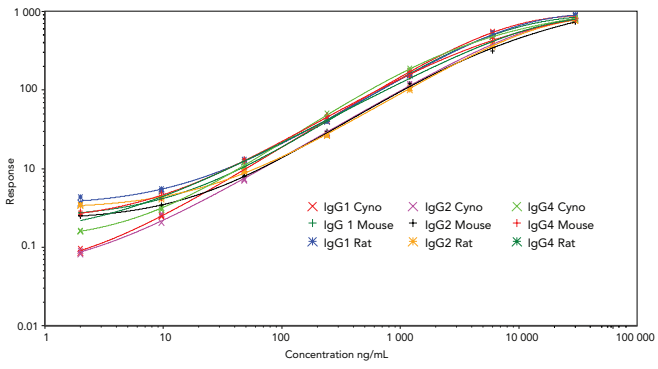

Figure 4. Specificity of human IgG 1, IgG2, and IgG 4 in neat cynomolgus and mouse serum using Gyrolab Generic PK Kit

High dilutional linearity and parallelism

Figure 5 shows results for Trastuzumab (Herceptin®), a humanized anti-HER2 receptor monoclonal antibody of IgG1 subtype, that was analyzed in 5, 10 and 20% pooled cynomolgus monkey serum and compared to 10% in buffer using Gyrolab Generic PK Kit. The optimal serum concentration was determined to be 10%. The data shows excellent linearity and parallelism over a large working range. Table 4 shows data from tests with 200 000 ng/mL of Denosumab (Prolia®), a human monoclonal antibody of IgG2 subtype, spiked in 100% cynomolgus monkey serum using Gyrolab Generic PK Kit. The results indicate excellent dilutional linearity for dilutions in the 200–8000x range.

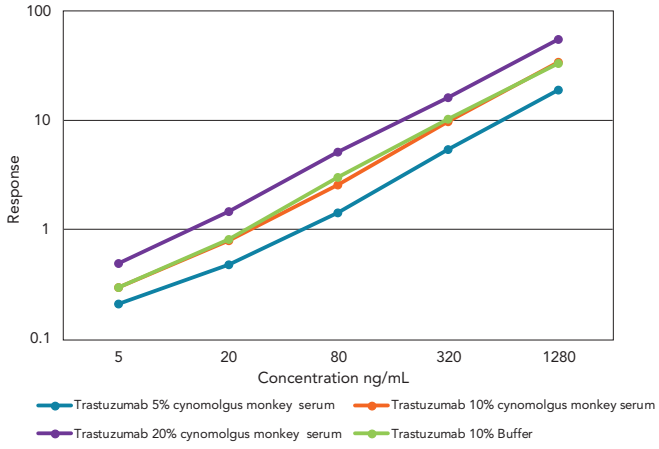

Figure 5. The optimal serum concentration was determined by preparing Trastuzumab in several concentrations of pooled cynomolgus monkey serum. Data kindly supplied by Eurofins Scientific.

Table 4. Dilutional linearity for Denosumab (Prolia®) in the range of 200-8000X.

Sample Id	Expected conc (ng/mL)	Dilution	n	Mean	SD	%CV	%RE	%TE
LQC Prolia	25	8000	3	25	1.4	5.5	1.8	7.3
MQC Prolia	250	800	3	205	16	7.6	-17.9	25.5
HQC Prolia	1000	200	3	866	76	9	-13	22.2

Ordering Information

Product number	Product name
P0020499	Gyrolab Generic PK Kit (for content, see below).
P0020500	Gyrolab Generic TK Kit (for content, see below).
P0020617	Gyrolab Generic PK CD50 Type E Kit (the kit contains reagents and consumables for 4800 datapoints).
P0020619	Gyrolab Generic PK CD50 Type F Kit (the kit contains reagents and consumables for 4800 datapoints).
P0020806	Gyrolab Generic TK CD50 Type E Kit (the kit contains reagents and consumables for 5600 datapoints).
P0020808	Gyrolab Generic TK CD50 Type F Kit (the kit contains reagents and consumables for 5600 datapoints).

Gyrolab Generic PK/TK Kits contents

	Quantity
Gyrolab Generic PK/TK Kit reagents	1 of each (see contents below)
Gyrolab Bioaffy 1000 HC (PK) or Gyrolab Bioaffy 20 HC (TK)	1
PCR Plate 96	3
Microplate foil	3
Gyrolab Wash station solution 2	1
Instructions for use	1

Note that standard is NOT included in kit.

Gyrolab Generic PK/TK Kit Reagents

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	Sample dilution buffer
Reagent F	Alternative sample dilution buffer Primarily for molecules with pI > 8

Storage conditions

Gyrolab Bioaffy 1000 HC and Gyrolab Bioaffy 20 HC CDs

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

Shelf life (unopened package): Minimum 12 months after delivery.

Gyrolab Generic PK/TK Kit Reagents

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

Shelf life (unopened package): see product label.

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