GYROS PROTEIN Technologies

Gyrolab® Assays

Simponi® (golimumab) PK Assay

INTRODUCTION

Simponi (golimumab) is an anti-inflammatory biopharmaceutical that belongs to the group of TNF-α (tumor necrosis factor-alpha) inhibitors. This monoclonal antibody was approved for treatment of rheumatoid arthritis in the European Union in October 2009.

To support clinical PK studies, we have developed a three-step sandwich Gyrolab PK Assay to determine Simponi in human serum samples. An MRD of 1:500 gives a broad analytical range with and approximate LLOQ of 200 ng/mL, and ULOQ of 300 µg/mL in neat serum. Use of this protocol on Gyrolab systems will reduce time to market and increase productivity while maintaining quality requirements.

ASSAY DESIGN

The assay was set up as a three-step sandwich assay with biotinylated human TNFα as a capture molecule and a mouse anti-human IgG Fc fragment as a detection molecule.

Mouse anti-human IgG Fc

Simponi

hTNFα

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust three-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 200 ng/mL to 300 µg/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in duplicate in three runs, was <20% (Table 2).

Table 1 Estimated Assay Range in neat matrix, based on three runs

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 200	~ 300 000

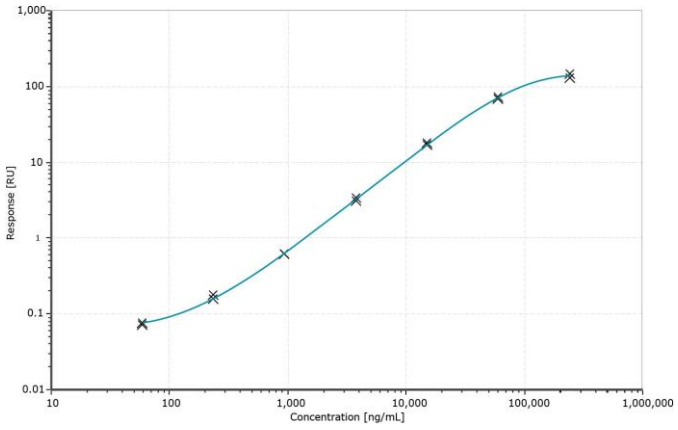

Figure 1 Standard curve in 0.5% serum. Concentrations in neat serum

Table 2 Accuracy and precision data of QC samples in neat matrix, n = number of runs

QC	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	Inter Run CV% (n=3)	Average Intra Run CV% (n=3)	Average Total Error% (n=3)
LLOQ	200	212	14	14	21
LQC	500	543	13	6.9	21
MQC	100000	94767	5.5	5.6	11
HQC	250000	228667	10	7.6	16
ULOQ	300000	308500	9.5	9.7	13

Selectivity

Selectivity was established by spiking 200 ng/mL of the drug in human serum samples. All samples measured <LLOQ when analyzed unspiked.

Table 3 Selectivity spiked samples

Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV%	Average Bias%
1	200	223	21	12
2	200	211	5.5	5.5
3	200	316	9.2	58
4	200	251	3.1	25
5	200	227	0.98	13
6	200	219	3.9	10
7	200	252	27	26
8	200	192	24	-3.9
9	200	241	0.093	20
10	200	201	4.5	0.49

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step format with two wash solutions (1000-3W-006-A). The assay buffer was Rexxip H with an MRD of 1:500. hTNFα from Prospec was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 70 nM and 630 nM biotinylated BSA (B-2007) from Vector Laboratories diluted in PBST.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the mouse anti-human IgG Fc JDC-10 from Southern Biotech, diluted to 5 nM in Rexxip F. The assay standard used was the human IgG1 monoclonal antibody golimumab, from Janssen Biologics. The standard was diluted in Rexxip H containing 0.2% human serum pool, Seralab.

Recommendations

When developing this assay for a specific drug development purpose, it is important to screen matrices and assess backgrounds, in particular for the specific disease matrices. Parameters, such as LLOQ need to be validated in-house. Data given in this document should only be considered as a guidance.

For additional support contact your local Field Application Support