GYROS PROTEIN Technologies

Gyrolab® Assays

Humira® (adalimumab) PK Assay

INTRODUCTION

Humira (adalimumab) is a tumor necrosis factor-alpha (TNF-α) inhibitor. TNF inhibitors are effective in treating autoimmune diseases. Adalimumab was the first fully human monoclonal antibody approved by the U.S. Food and Drug Administration.

To support clinical PK studies, we have developed a three-step sandwich Gyrolab PK Assay to determine Humira 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 300 000 ng/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

Humira

Human TNF-α

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 300 000 ng/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in duplicate in 3 runs, was <20% (Table 2).

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

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

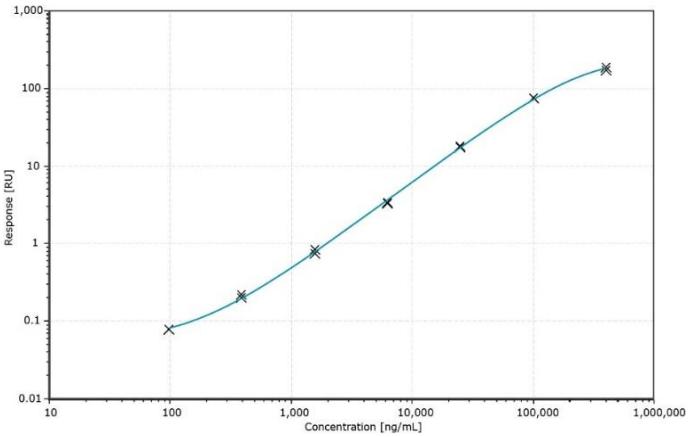

Figure 1 Standard curve in 0.5% serum. Concentrations in neat serum

Table 2 Accuracy and precision data of QC samples in neat serum, 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	173	9.4	7.9	22
LQC	500	489	7.3	5.9	6.7
MQC	10000	110833	4.1	3.2	9.4
HQC	250000	252167	0.2	4.7	13
ULOQ	300000	319167	6.0	4.4	15

Selectivity

Selectivity was established by spiking 200 ng/mL of the drug in human serum samples.

Table 3 Selectivity spiked samples.

Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV%	Average Bias%
1	200	211	8.5	5.6
2	200	244	11	22
3	200	176	6.7	-12
4	200	209	24	4.3
5	200	211	13	5.7
6	200	251	3.3	26
7	200	188	19	-6.1
8	200	208	3.5	4.2
9	200	163	10	-18
10	200	276	2.5	38

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using a Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step Gyrolab method with two wash solutions (1000-3W-006-A). The assay buffer used was Rexxip H with an MRD of 1:500. hTNFa (CYT-223) from Prospec was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 210 nM and 490 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 6.25 nM in Rexxip F. The assay standard used was the recombinant human IgG1κ monoclonal antibody adalimumab, targeting TNFα, from Abbvie. 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