GYROS PROTEIN Technologies

Gyrolab® Assays

Humira® (Adalimumab) Bridging PK Assay

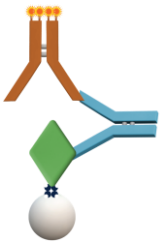
INTRODUCTION

Humira (Adalimumab) is an anti-inflammatory biopharmaceutical that belongs to the group of TNF-α (tumor necrosis factor-alpha) inhibitors. Adalimumab was the first fully human monoclonal antibody approved by the U.S. Food and Drug Administration.

We have developed a three-step bridging Gyrolab PK Assay to determine Humira in human serum samples. The use of a bridging format allows for lower concentrations of analyte to be measured and greatly reduces matrix interference. An MRD of 2 gives a broad analytical range with an approximate LLOQ of 20 ng/mL, and ULOQ of 22 000 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 bridging assay with biotinylated TNFα as a capture molecule and an anti-idiotype against Adalimumab as a detection molecule.



Anti-Adalimumab

Humira

TNFα

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 4-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 20 ng/mL to 22 000 ng/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicate in 3 runs, was <20% (Table 2).

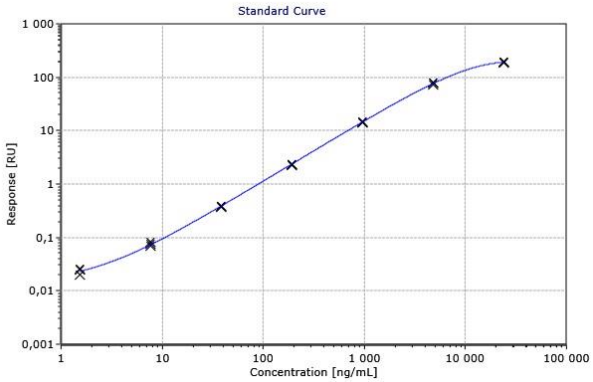


Figure 1 Standard curve in 50 % serum. Concentrations in neat serum.

Table 1 Estimated Assay Range in neat serum, based on 3 runs

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 20	~ 22 000

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	20	22	6.2	5.7	15
LQC	50	50	2.5	2.6	3.6
MQC	200	189	3.5	2.6	8.2
HQC	16 000	15 027	3.7	3.3	9.4
ULOQ	22 000	20 093	5.8	5.7	14

Selectivity

Selectivity was established by spiking 20 ng/mL of the drug in neat human serum samples, and then diluting twofold in Rexxip H-max. 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	20	22	5.1	11
2	20	21	5.4	7.0
3	20	23	4.1	17
4	20	23	6.1	17
5	20	24	3.2	18
6	20	22	7.8	8.0
7	20	23	4.7	15
8	20	22	7.4	8.9
9	20	22	4.5	10
10	20	22	12	8.6

MATERIALS AND METHODS

The assay was developed on Gyrolab xPlore using Gyrolab Bioaffy 200 CD. The assay was set up using a three-step assay with two wash solutions (200-3W-002-A). The assay buffer was Rexxip H-max with an MRD of 2. TNFα (CYT-223, ProSpec) was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and mixed with biotinylated BSA (B-2007, Vector Laboratories) to form the capture reagent. The concentration of biotin-TNFα was 50 nM and the concentration of biotin-BSA was 950 nM.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was an anti-idiotype against Adalimumab (clone 972557, R&D Systems), diluted to 12.5 nM in Rexxip F. The assay standard used was the Humira from AbbVie. The standard was prepared in neat human serum and diluted in Rexxip H-max before being loaded onto the microtiter plate.

Summary table

Capture	50 nM Biotinylated TNFα (CYT-223, ProSpec) + 950 nM biotinylated BSA (B-2007, Vector Laboratories)
Detection	Alexa Fluor 647 labeled anti-Adalimumab (clone: 972557, R&D Systems) 12.5 nM in Rexxip F
Analyte	Humira (AbbVie) in Rexxip H-max with 50% human serum
CD-type	Bioaffy 200 CD
Method	200-3W-002-A
Wash buffer for needles	Wash buffer 1: PBS-T, wash buffer 2: Gyrolab wash buffer pH 11
PMT-setting	1%
Expected dynamic range	Approximately 20-22 000 ng/mL in neat serum. Please note that the range can be shifted by using a different CD. The Bioaffy 1000 CD will give more sensitivity and Bioaffy 20 HC will give a higher ULOQ.

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