GYROS PROTEIN Technologies

Gyrolab® Assays

Enbrel® (enteracept) PK Assay

INTRODUCTION

Enbrel (enteracept) is an anti-inflammatory biopharmaceutical that belongs to the group of TNF-α (tumor necrosis factor-alpha) inhibitors. Etanercept is a fusion protein consisting of the ligand-binding part of a human TNF receptor 2, coupled to the Fc domain of human IgG1.

To support clinical PK studies, we have developed a three-step sandwich Gyrolab PK Assay to determine Enbrel 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 100 000 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 TNF-α as a capture molecule and a mouse anti-human IgG Fc Fragment as a detection molecule.

Mouse anti-human IgG Fc

Enbrel

hTNF-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 100 000 ng/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in duplicates in three runs, was <20% (Table 2).

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

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 200	~ 100 000

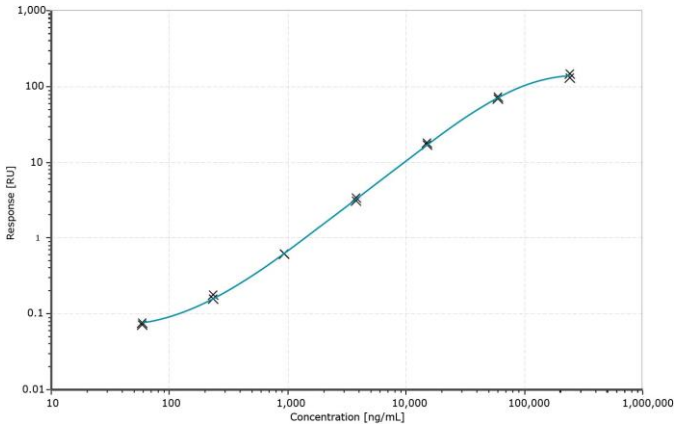

Figure 1 Standard curve in 0.5% serum. Concentrations in neat serum

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	223	8.4	6.0	17
LQC	500	511	3.1	3.0	5.2
MQC	40000	38733	3.0	2.3	5.5
HQC	80000	91783	5.2	3.6	19
ULOQ	100000	94450	8.5	7.7	13

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	206	8.9	2.8
2	200	207	0.8	3.4
3	200	213	3.2	6.5
4	200	173	7.0	-13
5	200	189	1.2	-5.7
6	200	192	7.9	-4.1
7	200	232	4.1	16
8	200	219	15	9.4
9	200	200	4.7	0.2
10	200	225	10	13

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using 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 was Rexxip H with an MRD of 1:500. hTNFα (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 dimeric fusion Fc-protein enteracept, targeting TNFα, from Pfizer. The standard was prepared 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