GYROS PROTEIN Technologies

Gyrolab® Assays

Kineret® (anakinra) PK Assay

INTRODUCTION

Kineret (anakinra) is an anti-inflammatory biopharmaceutical drug used to treat rheumatoid arthritis and similar inflammatory diseases. It is a recombinant modified version of the human interleukin 1 receptor antagonist (IL-1 RA) protein and works by blocking the action of IL-1.

We have developed a three-step bridging Gyrolab PK Assay to determine Kineret in human serum samples. An MRD of 1:10 gives a broad analytical range with an approximate LLOQ of 50 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 bridging assay with biotinylated polyclonal anti-IL-1 RA as a capture molecule and monoclonal anti-IL-1 RA labeled with Alexa Fluor® 647 as a detection molecule.

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 3-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 50 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 triplicate in three runs, was <20% (Table 2).

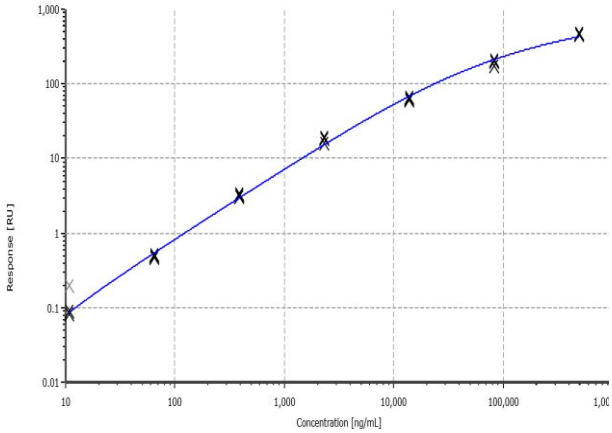

Figure 1 Standard curve in Rexxip H with 10% serum. Concentrations in neat serum

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

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 50	~ 100 000

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)
1	50	48	14	3.5	13
2	400	474	14	5.0	23
3	2 500	2 936	11	3.7	21
4	15 000	15 500	18	17	26
5	100 000	109 191	10	6.2	18

Selectivity

Selectivity was established by spiking 60 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	60	58	7.6	-3.8
2	60	71	11	18
3	60	64	5.7	7.3
4	60	61	9.6	1.0
5	60	59	9.2	-1.6
6	60	62	5.9	2.5
7	60	60	4.2	0
8	60	64	2.6	7.0
9	60	58	5.3	-3.6
10	60	56	2.6	-6.5

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step Gyrolab method with two wash solutions (1000-3W-006-A) and a 1% PMT setting. The assay buffer was Rexxip H with 10% human serum. Anti-human IL-1 RA from BioRad (AHP2184B) was supplied biotinylated and used at a concentration of 700 nM, diluted with PBS-T.

The detection antibody labeled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was monoclonal anti-IL-1 RA (BioRad, VMA00500), diluted to 35 nM in Rexxip F. The assay standard was the recombinant IL-1 RA anakinra from Swedish Orphan Biovitrium (Sobi). The standard was prepared in 10% human serum diluted in Rexxip H.

Summary table

Capture	700 nM biotinylated polyclonal anti-IL1 RA (AHP2184B, BioRad), in PBS-T
Detection	Alexa Fluor 647 labeled monoclonal anti-IL1 RA (VMA00500, BioRad) 35 nM in Rexxip F
Analyte	Kineret (Sobi) in Rexxip H with 10% human serum
CD-type	Bioaffy 1000 CD
Method	1000-3W-006-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 50-100 000 ng/mL in neat human serum

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 should be validated in-house. Data given in this document should only be considered as guidance.

For additional support contact your local Field Application Support