GYROS PROTEIN Technologies

Gyrolab® Assays

Orencia® (abatacept) PK Assay

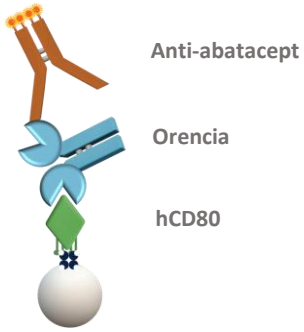
INTRODUCTION

Orencia (abatacept) is an immunosuppressive biopharmaceutical used to treat autoimmune disease. Orencia is a fusion protein composed of the Fc region of IgG1 fused to the extracellular region of CTLA-4. Orencia binds to CD80 and CD86, preventing activation of T lymphocytes.

We have developed a three-step bridging Gyrolab PK assay to determine Orencia in human serum samples. An MRD of 1:20 gives a broad analytical range with an approximate LLOQ of 150 ng/mL, and ULOQ of 200 000 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 human CD80 as a capture molecule and mouse anti-abatacept labeled with Alexa Fluor® 647 labeled 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 150 ng/mL to 200 000 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).

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

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 150	~ 200 000

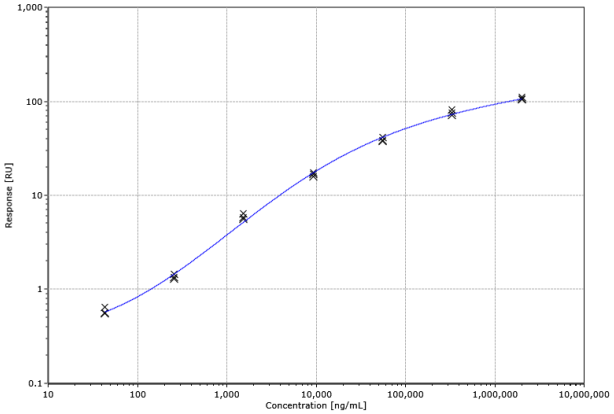


Figure 1 Standard curve in Rexxip H with 5% serum. Concentrations in neat serum.

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)
1	150	128	11	12	27
2	400	382	12	7.6	16
3	2 000	2 341	7.9	5.1	22
4	200 000	180 670	11	10	18

Selectivity

Selectivity was established by spiking 150 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	150	183	5.0	22
2	150	149	8.5	-0.71
3	150	145	3.7	-3.0
4	150	159	7.4	6.1
5	150	173	7.6	15
6	150	163	8.4	8.4
7	150	133	2.9	-11
8	150	184	7.6	23
9	150	171	6.7	14
10	150	167	6.7	12

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 5% PMT setting. The assay buffer was Rexxip H with 5% human serum. Human CD80 from Abcam (ab180050) was biotinylated as detailed below and used at a concentration of 700 nM, diluted with PBS-T.

Recombinant CD80 protein as supplied was reconstituted to 1 mg/mL with deionized water. The reconstituted protein was then reacted with 12 molar equivalents of Biotin-XX, SE (Thermo Fisher Scientific, B1606) as a 4 mg/mL solution freshly prepared using DMSO, and the resulting mixture vortexed briefly, before being roller-mixed for 1 hour at 20°C in the dark. The conjugate was purified by desalting into 50 mM phosphate 150 mM NaCl pH 6.7 buffer via Zeba 0.5 mL spin desalting column, and UV analyzed to determine product concentration.

The detection antibody, labeled with Alexa Fluor 647 as detailed below, was mouse anti-abatacept DDX5040 from Dendritics, diluted to 35 nM in Rexxip F. The assay standard used was the fusion protein abatacept from Bristol-Myers Squibb. The standard was prepared in 5% human serum diluted in Rexxip H.

Anti-abatacept antibody as supplied was desalted into 100 mM carbonate pH 8.5 buffer via Zeba 2 mL spin desalting column (Thermo Fisher Scientific, 89890), and then UV analyzed to determine concentration. The desalted antibody was then reacted with 20 molar equivalents of AF647-SE (Thermo Fisher Scientific, A37573) as a 1 mg/mL solution freshly prepared using DMSO, and the resulting mixture vortexed briefly, then roller-mixed for 1 hour at 20°C in the dark. The conjugate was purified by desalting into 50 mM phosphate 150 mM NaCl pH 6.7 buffer via Zeba 2 mL spin desalting column, and UV analyzed to determine product concentration.

Summary table

Capture	700 nM biotinylated CD80 (ab180050, Abcam)
Detection	Alexa Fluor 647 labeled anti-abatacept (DDX5040, Dendritics) 35 nM in Rexxip F
Analyte	Orencia (Bristol-Myers Squibb) in Rexxip H with 5% 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	5%
Expected dynamic range	Approximately 150-200 000 ng/mL in neat matrix

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