GYROS PROTEIN Technologies

Gyrolab® Assays

Opdivo® (nivolumab) PK Assay

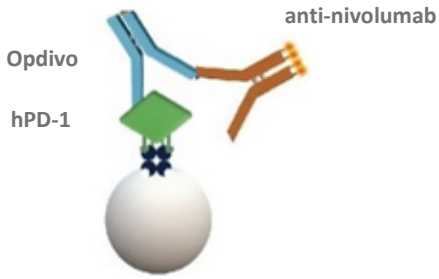
INTRODUCTION

Opdivo (nivolumab) is a cancer immunotherapy biopharmaceutical that belongs to the group of PD-1 (programmed cell death protein 1) inhibitors. Opdivo is a humanized antibody of IgG4 isotype that blocks a protective mechanism of cancer cells, allowing the immune system to destroy them.

We have developed a three-step bridging Gyrolab PK Assay to determine Opdivo in human serum samples. An MRD of 1:20 gives a broad analytical range with an approximate LLOQ of 40 ng/mL, and ULOQ of 18 000 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 PD-1 as a capture molecule and monoclonal recombinant human anti-nivolumab 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 40 ng/mL to 18 000 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)
~ 40	~ 18 000

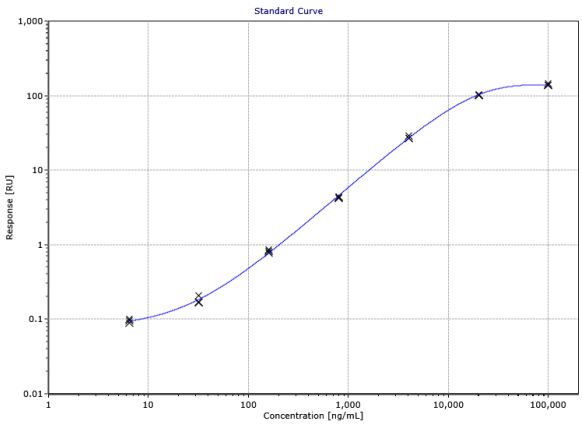


Figure 1 Standard curve in Rexxip H with 5% serum. Concentrations in neat serum

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	40	40	13	14	18
2	200	202	4.2	3.8	5.1
3	1 000	954	4.6	3.9	8.4
4	3 000	3 108	4.8	3.7	7.3
5	18 000	18 735	8.3	2.3	11

Selectivity

Selectivity was established by spiking 40 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	40	41	12	3.2
2	40	40	11	0.66
3	40	39	9.3	-3.1
4	40	34	14	-15
5	40	39	0.73	-2.1
6	40	47	16	17
7	40	49	15	22
8	40	43	19	6.5
9	40	41	15	1.7
10	40	37	8.2	-7.5

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 5% PMT setting. The assay buffer was Rexxip H with 5% human serum. Human PD-1 from Abcam (ab174035) was biotinylated as detailed below and used at a concentration of 350 nM, diluted with PBS-T containing 350 nM Biotin-BSA .

Recombinant PD-1 protein and BSA (sigma, A3808) 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 was labeled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide). The recombinant human anti-nivolumab HCA301 from BioRad was diluted to 35 nM in Rexxip F. The assay standard used was the humanized IgG4 targetting PD-1 nivolumab from Bristol-Myers Squibb. The standard was prepared in 5% human serum diluted in Rexxip H.

Summary table

Capture	350 nM biotinylated PD-1 (Abcam, ab174035) + 350 nM biotinylated BSA in PBS-T
Detection	Alexa Fluor 647 labeled anti-nivolumab (clone AbD30258_hIgG1, BioRad, HCA301), 35 nM in Rexxip F
Analyte	Opdivo (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 40-18 000 ng/mL in neat 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