GYROS PROTEIN Technologies

Gyrolab® Assays

Keytruda® (pembrolizumab) PK Assay

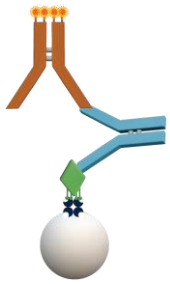
INTRODUCTION

Keytruda® (pembrolizumab) is a cancer immunotherapy biopharmaceutical that belongs to the group of PD-1 (programmed cell death protein 1) inhibitors. Keytruda 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 bridging three-step sandwich Gyrolab PK assay to determine Keytruda in human serum samples. A Minimum Required Dilution (MRD) of 4 gives a broad analytical range with an LLOQ on the Gyrolab® Bioaffy™1000 CD of 18 ng/mL, and an LLOQ on the Gyrolab® Bioaffy™ 4000 CD of 6 ng/mL in neat serum. On both CD types the ULOQ was set at 6 000 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 bridging three-step sandwich assay with biotinylated human PD-1 as a capture molecule and a recombinant human anti-pembrolizumab labeled with Alexa Fluor® 647 as a detection molecule.



Anti-pembrolizumab

Keytruda

hPD-1

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

Robust, wide range standard curves (Figure 1) were generated over three runs each using two different Gyrolab Bioaffy CDs, achieving the assay ranges shown in Table 1. The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in triplicate in three runs on each CD type, was <20% (Table 2) for both CD types.

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

CD Type	LLOQ (ng/mL)	ULOQ (ng/mL)
Gyrolab Bioaffy 1000	~ 18	~ 6 000
Gyrolab Bioaffy 4000	~ 6	~ 6 000

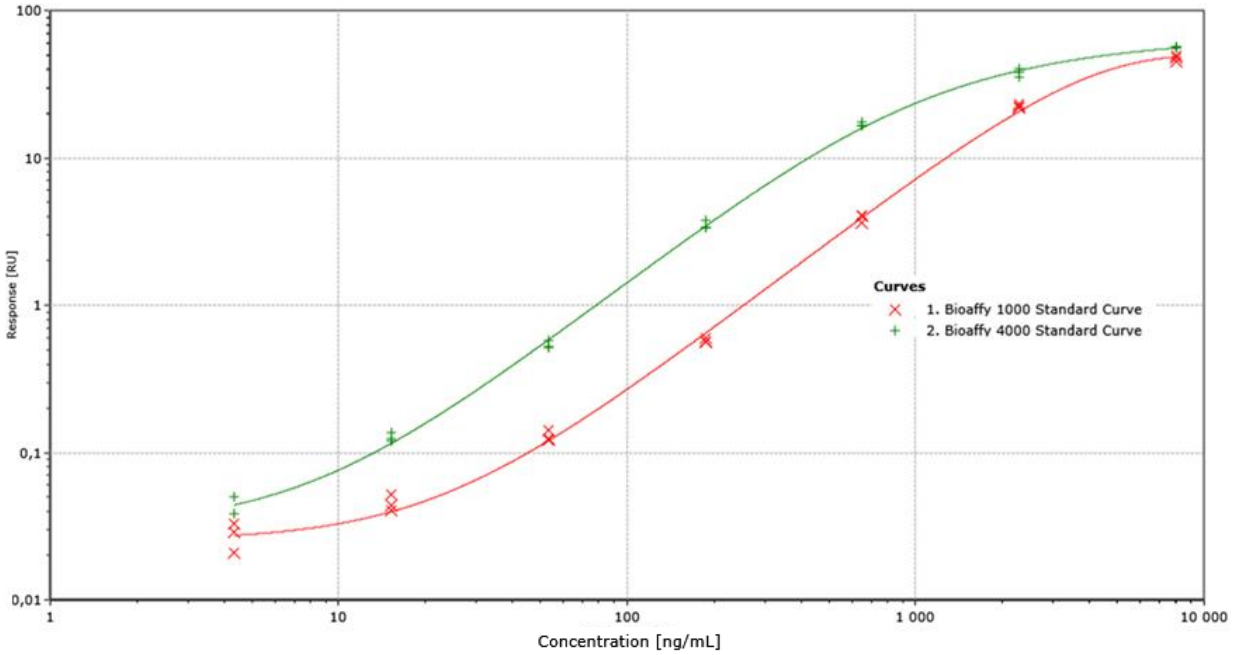


Figure 1 Standard curves in Rexxip H with 25% serum. Concentrations in neat serum

Table 2 Accuracy and precision data of QC samples in neat serum, n = number of runs

CD Type	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)
Gyrolab Bioaffy 1000	18	20	7.9	9.0	20
	54	61	4.6	5.8	18
	200	200	13	12	22
	2 000	2 200	7.8	2.8	14
	6 000	5 600	6.9	12	19
Gyrolab Bioaffy 4000	6	7.1	20	12	31
	18	18	9.8	6.6	16
	200	220	12	3.9	16
	2 000	2 100	11	6.1	17
	6 000	5 900	4.3	9.0	12

Selectivity

Selectivity was established by spiking Keytruda into human serum samples at the LLOQ level. All samples measured <LLOQ when analyzed unspiked.

Table 3 Selectivity spiked samples

CD Type	Sample	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	CV (%)	Average Bias (%)
Gyrolab Bioaffy 1000	1	18	20	6.6	12
	2	18	18	15	-2.3
	3	18	22	4.3	23
	4	18	22	8.7	22
	5	18	22	5.2	20
	6	18	18	5.7	-0.6
	7	18	25	6.0	37
	8	18	22	13	20
	9	18	18	11	-0.2
	10	18	22	7.2	22
Gyrolab Bioaffy 4000	1	6	7.2	11	20
	2	6	5.7	22	-4.9
	3	6	6.9	-	15
	4	6	6.4	9.4	7.2
	5	6	4.6	1.9	-23
	6	6	6.1	12	2.5
	7	6	5.0	15	-16
	8	6	5.6	-	-7.4
	9	6	6.4	16	7.3
	10	6	6.8	15	13

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab Bioaffy 1000 CD and Gyrolab Bioaffy 4000 CD. The assay was set up using 3-step Gyrolab methods with two wash solutions (1000-3W-006-A and 4000-3W-001-A) and a 1% PMT setting. The assay buffer was Rexxip H with 25% human serum. Human PD-1 from Abcam (ab174035) was biotinylated as detailed below and used in a concentration of 350 nM with 350 nM Biotin-BSA diluted with PBS-T.

Recombinant PD-1 protein was reconstituted in 500 µL deionized water, to a concentration of 200 µg/mL. The reconstituted protein was then was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide), with a 1:5 challenge ratio of biotin.

The detection antibody, labeled with Alexa Fluor® 647 as detailed below, was recombinant human anti-pembrolizumab HCA297 from Bio-Rad diluted to 35 nM in Rexxip F. The assay standard used was humanized IgG4 monoclonal antibody pembrolizumab from MSD. The standard was prepared in 25% human serum diluted in Rexxip H.

Anti-pembrolizumab antibody as supplied was desalted into 100 mM carbonate pH 8.5 buffer via Zeba 2 mL spin desalting column 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	350 nM biotinylated PD-1 (ab174035, Abcam) + 350 nM biotinylated BSA (A3803, Sigma-Aldrich) in PBS-T	
Detection	Alexa Fluor 647 labeled anti-pembrolizumab (HCA297, Bio-Rad), 35 nM in Rexxip F	
Analyte	Keytruda (MSD) in Rexxip H with 25% human serum	
CD-type	Gyrolab® Bioaffy™ 1000 CD	Gyrolab® Bioaffy™ 4000 CD
Method	1000-3W-006-A	4000-3W-001-A
Full CD run time	Approx. 70 minutes	Approx. 90 minutes
Sample volume required (triplicate)	10 µL	16 µL
Expected dynamic range	Approximately 18 – 6 000 ng/mL in neat serum	Approximately 6 – 6 000 ng/mL in neat serum
Minimum required dilution	1-in-4	
Wash buffer for needles	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11	
PMT-setting	1%	

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