

Gyrolab® Assays

# Atezolizumab PK Assay

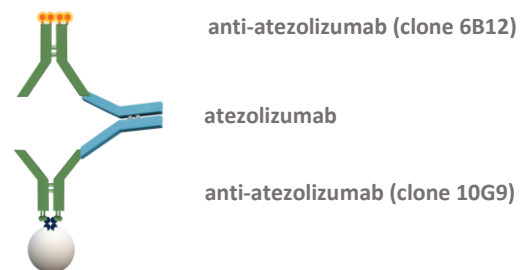
## INTRODUCTION

Atezolizumab (marketed under the trade name Tecentriq®) is a humanized monoclonal antibody of IgG1 isotype against the protein programmed cell death-ligand 1 (PD-L1). Atezolizumab blocks the interaction of PD-L1 with programmed cell death protein 1 (PD-1) and CD80 receptors. PD-L1 can be highly expressed on tumors, which is thought to lead to reduced activation of immune cells (cytotoxic T-cells in particular) that might otherwise recognize and attack the cancer. Inhibition of PD-L1 by Atezolizumab can remove this inhibitor effect and thereby engender an anti-tumor response.

We have developed a three-step bridging Gyrolab PK assay to determine the level of atezolizumab in human serum samples. An MRD of 1:4 gives a broad analytical range with an approximate LLOQ of 5 ng/mL, and ULOQ of 15,000 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 anti-attezolizumab (clone 10G9) as a capture molecule and anti-attezolizumab (clone 6B12) labeled with Alexa Fluor® 647 as a detection molecule.



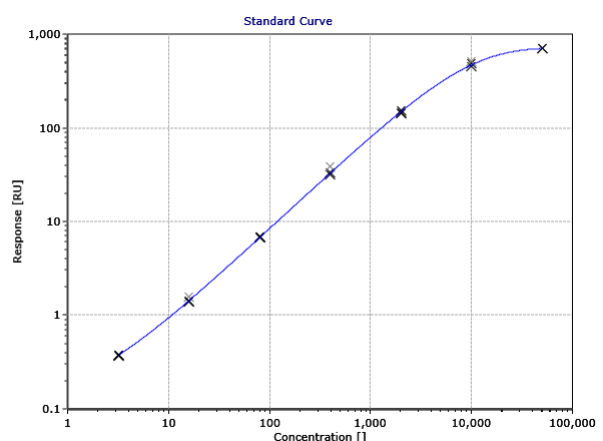
## ASSAY PERFORMANCE

### Dynamic range, accuracy and precision

A robust 3-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 5 ng/mL to 15 000 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)
~ 5	~ 15 000



**Figure 1** Standard curve in Rexpix H with 20% 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	5.0	5.8	4.3	9.7	25
2	30	33	7.6	7.4	17
3	250	259	13	2.1	11
4	1 500	1 604	8.4	2.8	12
5	15 000	17 290	10	3.4	19

### Selectivity

Selectivity was established by spiking 5 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	5	5.8	7.2	16
2	5	4.6	8.9	-7.4
3	5	5.2	1.1	4.7
4	5	5.6	2.1	12
5	5	4.9	3.6	-2.1
6	5	4.4	11	-11
7	5	5.4	6.6	8.9
8	5	5.1	4.2	1.7
9	5	4.7	4.0	-5.4
10	5	5.5	5.7	9.2

## 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 20% serum. Anti-atezolizumab from GenScript (clone 10G9) was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in 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 anti-atezolizumab (clone 6B12) from GenScript, diluted to 35 nM in REXXIP F. The assay standard used was the atezolizumab (anti-PD-L1) from Stratech (A2004-SEL). The standard was prepared in human serum diluted in REXXIP H.

## Summary table

<b>Capture</b>	700 nM biotinylated anti-atezolizumab (A01948, GenScript clone 10G9), diluted in PBS-T
<b>Detection</b>	Alexa Fluor 647 labeled anti-atezolizumab (A01949, GenScript clone 6B12) 35 nM in Rexpip F
<b>Analyte</b>	Atezolizumab (A2004-SEL Stratech) in Rexpip H with 20% serum
<b>CD-type</b>	Bioaffy 1000 CD
<b>Method</b>	1000-3W-006-A
<b>Wash buffer for needles</b>	Wash buffer 1: PBS-T Wash buffer 2: Gyrolab Wash Buffer pH 11
<b>PMT-setting</b>	1%
<b>Expected dynamic range</b>	Approximately 5-15 000 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

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