GYROS PROTEIN Technologies

Gyrolab® Assays

Avastin® (bevacizumab) PK Assay

INTRODUCTION

Avastin® (bevacizumab) is a humanized anti-VEGF monoclonal IgG1 antibody, approved for the treatment of advanced colorectal cancer, advanced non-small cell lung cancer, metastatic breast cancer and advanced renal cell cancer. Avastin is designed to directly bind to VEGF extracellularly to prevent interaction with VEGF receptors (VEGFRs) on the surface of endothelial cells, and thereby may inhibit VEGF's angiogenic activity.

We have developed a three-step sandwich Gyrolab PK Assay to determine Avastin in serum samples. An MRD of 20 gives a broad analytical range with and approximate LLOQ of 50 ng/mL, and ULOQ of 10 000 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 sandwich assay with biotinylated human VEGF165 as a capture molecule and an Alexa Fluor® 647 labelled anti-bevacizumab/anti-ranibizumab detection molecule.

anti-bevacizumab antibody

Avastin

hVEGF165

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 10 000 ng/mL (Table 1). The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in 3-plicate in 3 runs, was <20% (Table 2).

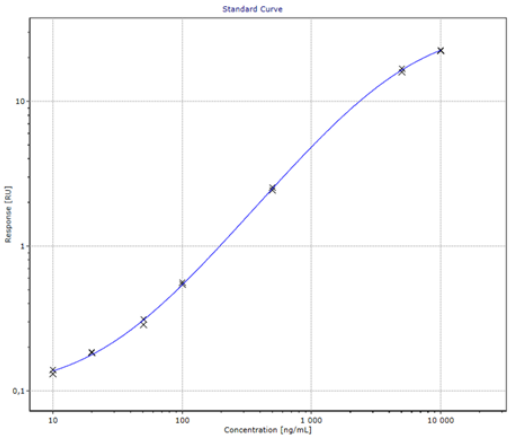

Figure 1 Standard curve in 5% human serum. Concentrations in neat human serum

Table 1 Estimated assay range in neat human serum, based on 3 runs

LLOQ (ng/mL)	ULOQ (ng/mL)
~ 50	~ 10 000

Table 2 Accuracy and precision data of QC samples in neat human serum sample, 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)
LLOQ	50	54	15	10	23
LQC	150	142	4.0	3.5	8.6
MQC	500	488	7.9	3.2	10
HQC	6 000	6225	13	7.4	16
ULOQ	10 000	11424	7.1	6.5	21

Selectivity

Selectivity was established by spiking 50 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	50	25	13	-50
2	50	38	2.8	-24
3	50	42	9.8	-16
4	50	47	3.4	-6.9
5	50	50	4.8	-0.2
6	50	37	2.3	-25
7	50	39	1.7	-21
8	50	42	3.7	-17
9	50	41	11	-18
10	50	47	2.5	-6.1
pool	50	44	4.3	-11

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using Gyrolab Bioaffy 1000 CD. The assay was set up using a 3-step Gyrolab method with two wash solutions (1000-3W-006-A). The assay buffer was Rexxip HN with 5% human serum. Biotinylated human VEGF165, epitope tag free, was purchased from ACROBiosystems and used in a concentration of 526 nM (10 µg/mL), diluted with 1% BSA in PBST.

The detection antibody, labeled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the human anti-Ranibizumab antibody, clone AbD29928 from Bio-Rad, diluted to 3 nM in Rexxip F. The assay standard used was Avastin from Roche. The standard was prepared in human serum diluted in Rexxip HN.

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

Summary table

Capture	Biotinylated human VEGF165, epitope tag free, ultra sensitivity (primary amine labeling), ACROBiosystems, VE5-H8210
Detection	human anti-Ranibizumab antibody, clone AbD29928, Bio-Rad, HCA304
Analyte	Avastin (25 mg/mL concentrate for solution for infusion), Roche
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 pH11
PMT-setting	1%
Expected dynamic range	50 – 10 000 ng/mL in neat serum

For additional support contact your local Field Application Support