

Gyrolab® Assays

Human VEGF Assay

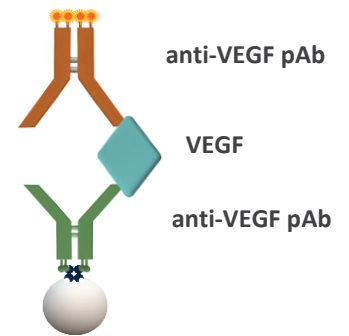
INTRODUCTION

Vascular endothelial growth factor (VEGF) is a protein expressed in a range of cell types and is a potent mediator in the formation of blood vessels.

We have developed a three-step sandwich Gyrolab assay to determine VEGF in human serum samples. 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 goat anti-human VEGF₁₆₅ antibody as a capture molecule and the same goat anti human-VEGF₁₆₅ antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant VEGF₁₆₅ was used as standard material



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 4 pg/mL to 1500 pg/mL (Table 1). The Limit of Detection (LOD) was determined as the concentration corresponding to at least two standard deviations above the assay blank.

The inter-run precision (CV, Coefficient of Variation), established with QC samples over the assay range run in duplicate in three runs, was <20% (Table 2).

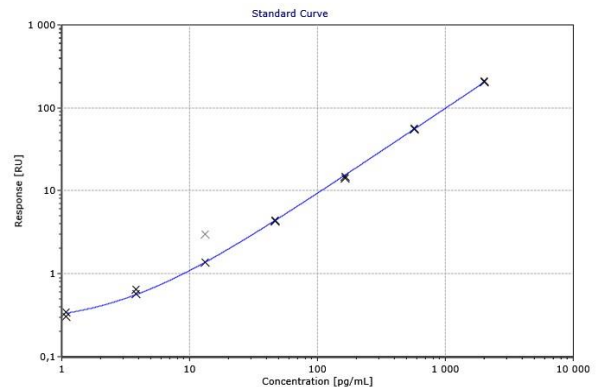


Figure 1 Standard curve in REXXIP® H

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 2.5	~ 4	~ 1500
In neat matrix	~ 5	~ 8	~ 3000

Table 2 Accuracy and precision data of QC samples in REXXIP H, n = number of runs

QC	Expected Conc (pg/mL)	Average Measured Conc (pg/mL)	Inter Run CV (%; n=3)	Average Intra Run CV (%; n=3)	Average Total Error (%; n=3)
1	4.0	4.3	14	8.5	18
2	6.0	6.3	4.3	3.5	7.8
3	100	98	5.7	6.2	9.9
4	1000	981	6.8	6.5	11
5	1500	1507	2.2	1.9	3.4

Parallelism

Parallelism was examined by diluting human serum samples from two individuals and one serum pool containing detectable endogenous levels of VEGF. The samples were serially diluted in Rxxip H-max (1:2 dilution) and Rxxip H (1:4 - 1:64) (Table 3). It is recommended that the end user performs additional parallelism assessment on human serum samples with endogenous levels of VEGF.

Table 3 Parallelism. Each dilution analyzed in triplicate

Sample	Dilution Factor	Calculated Conc. (pg/mL)	CV (%)	Recovery % of lowest dilution
Human Serum Sample 1	2	199	2.9	100
	4	189	6.4	95
	8	190	2.2	96
	16	196	6.6	98
	32	195	-	98
	64	<LLOQ	-	-
Human Serum Sample 2	2	190	5.2	100
	4	185	2.3	97
	8	200	5.8	105
	16	193	6.3	102
	32	211	9.9	111
	64	<LLOQ	-	-
Human Serum Sample Pool	2	476	3.0	100
	4	461	5.7	97
	8	467	2.1	98
	16	470	7.2	99
	32	473	1.6	99
	64	506	4.6	106

MATERIALS AND METHODS

The assay was developed on a Gyrolab xP system using Gyrolab® Bioaffy™ 4000 CD. The assay was set up using a three-step Gyrolab method with two wash solutions (4000-3W-001-A) and a 5% PMT setting. The assay buffer was Rxxip H. The Minimum Required Dilution (MRD) for serum samples was 1:2. Goat polyclonal anti-human VEGF₁₆₅ antibody from R&D Systems was biotinylated according to the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL, diluted with PBS-T.

The detection antibody was a Goat polyclonal anti-human VEGF antibody from R&D systems labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide) and diluted to 50 nM in Rxxip F. Recombinant Human VEGF₁₆₅ (Acro biosystems) was used as assay standard. The standard curve was prepared in Rxxip H.

Summary table

Capture	Goat polyclonal anti-human VEGF ₁₆₅ antibody (R&D Systems, cat no. AF-293-NA), biotinylated and diluted to 100 µg/mL in PBS-T
Detection	Goat polyclonal anti-human VEGF ₁₆₅ antibody (R&D Systems, cat no. AF-293-NA), labeled with Alexa Fluor® 647, 50 nM in Rexasip® F
Analyte	Human VEGF ₁₆₅ Protein (Acro Biosystems, cat no. VE5-H4210) in Rexasip® H
CD-type	Gyrolab® Bioaffy™ 4000
Method	4000-3W-001-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 4 pg/mL to 1500 pg/mL (8 pg/mL to 3000 pg/mL in neat matrix, diluted 1:2)

Recommendations

When developing this assay, 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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