GYROS PROTEIN Technologies

Gyrolab® Assays

Total GLP-1 Assay

INTRODUCTION

Glucagon-Like Peptide 1 (GLP-1) is a peptide hormone of the glucagon family that is produced upon food consumption by the intestinal L-cells from the same prohormone as glucagon. GLP-1 (7-36) amide, the principle active form of GLP-1, is a potent stimulator of glucose-dependent insulin secretion. When secreted into the blood the active GLP-1 is rapidly cleaved to the metabolite GLP-1 (9-36) amide.

We have developed a three-step sandwich Gyrolab Assay to determine total GLP-1 (the sum of active GLP-1 and its metabolites) 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 a biotinylated anti-GLP-1 monoclonal antibody as a capture molecule and an anti-GLP-1 monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule.

anti-GLP-1 mAb

GLP-1 (7-36) amide

anti-GLP-1 mAb

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 8 pg/mL to 1 600 pg/mL (Table 1). The Limit of Detection (LOD) was determined as the concentration corresponding to at least two standards deviations above the assay blank.

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).

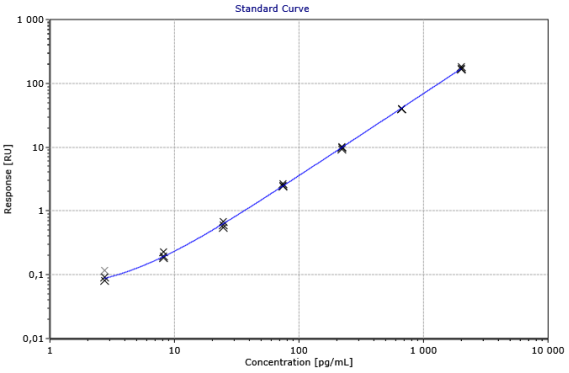

Figure 1 Standard curve in Rexxip HX

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~3	~8	~1 600
In neat matrix	~6	~16	3 200

Table 2 Accuracy and precision data of QC samples in Rexxip HX, 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)
LLOQ	8	9.1	14	4.2	22
LQC	20	21	12	4.1	16
MQC	80	77	13	2.8	12
HQC	1 000	902	13	5.1	16
ULOQ	1 600	1513	11	3.1	10

Dilution linearity

Linearity of dilution was examined by spiking GLP-1 (7-36) amide to ULOQ level into a human serum pool containing 0.1 mM diprotin A (Bachem, H-3825). The sample was serially diluted with Rexxip HX to obtain six data points (Table 3). This was done to set the MRD (1:2).

Table 3 Linearity of dilution

Dilution Factor	Calculated Conc (pg/mL)	Recovery (%)	CV (%)
2	1337	84	7.1
4	1367	85	0.6
8	1408	88	5.2
16	1430	89	5.7
32	1481	93	6.2
64	1530	96	7.1

Parallelism

Parallelism tests could not be performed since the endogenous level of total GLP-1 was below the quantification range. It is recommended that end users assess parallelism when suitable samples with significant levels of analyte are identified.

MATERIALS AND METHODS

The assay was developed on Gyrolab xP using Gyrolab Bioaffy 1000 CD. The assay was set up using a three-step method with two wash solutions (1000-3W-006-A) and a 5% PMT setting. The assay buffer was Rexxip HX with an MRD of 1:2. Anti-GLP-1 clone 5B10 from Novus Biologicals was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL, diluted with PBS-T.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the anti-GLP-1 clone 8G9 from Novus Biologicals, diluted to 5 nM in Rexxip F. The assay standard used was synthetic GLP-1 (7-36) amide, from Bachem (H-6795). The standard was prepared in Rexxip HX.

Summary table

Capture	100 µg/mL biotinylated anti-GLP-1 (clone 5B10, Novus Biologicals) in PBS-T
Detection	Alexa Fluor 647-labeled anti-GLP-1 (clone 8G9, Novus Biologicals), 5 nM in Rexxip F
Analyte	Synthetic GLP-1 (7-36) amide in Rexxip HX (Bachem, H6795)
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 8 pg/mL to 1 600 pg/mL (16 pg/mL to 3 200 pg/mL in neat human serum)

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 must be validated in-house. Data given in this document should only be considered as guidance.

For additional support contact your local Field Application Support