GYROS PROTEIN Technologies

Gyrolab® Assays

Active GLP-1 Assay

INTRODUCTION

Glucagon-Like Peptide 1 (GLP-1) is a peptide hormone of the glucagon family which 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.

We have developed a three-step sandwich Gyrolab Assay to determine active GLP-1 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 Alexa Fluor® 647 labelled anti-GLP-1 monoclonal antibody 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 3 pg/mL to 1 600 pg/mL (Table 1). The Limit of Detection (LOD) was determined as a 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).

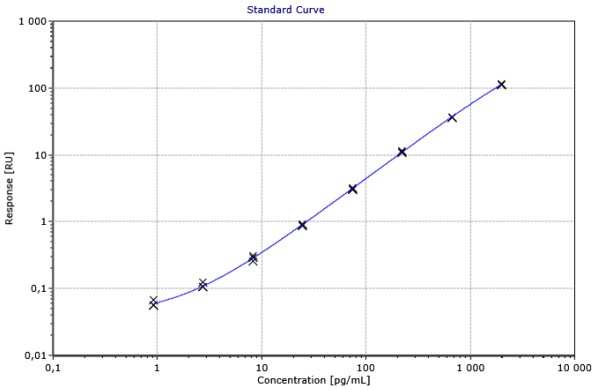

Figure 1 Standard curve in Rexxip HX

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~2	~3	~1 600
In neat matrix	~2	~3	~1 600

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	3	3.4	14	15	27
LQC	6	5.8	11	4.9	14
MQC	50	49	6.4	6.5	9.4
ULOQ/HQC	1 600	1 622	5.7	2.4	6.7

Dilution linearity

Linearity of dilution was examined by spiking GLP-1 (7-36) amide to ULOQ level into a human serum sample. The sample was serially diluted with Rexxip HX to obtain five data points.

Dilution Factor	Calculated	% Recovery	CV%
1	1 695	106	1.5
2	1 861	116	3.2
4	1 601	100	4.0
8	1 398	87	4.8
16	1 274	80	3.1

Parallelism

Parallelism tests could not be performed since endogenous level of active GLP-1 were below the quantification range. It is recommended that the end user performs parallelism assessment when suitable samples with significant levels of analyte are identified.

MATERIALS AND METHODS

The assay was developed on Gyrolab xPand and Gyrolab xP using Gyrolab Bioaffy 1000 CD. The assay was set up using a three- step method; 1000-3W-006-A. The assay buffer was Rexxip HX with an MRD of 1 (no dilution required). Anti-GLP-1 clone 10 from ThermoFisher Scientific 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 10, ThermoFisher Scientific) 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 pH11
PMT-setting	5%
Expected dynamic range	Approximate 3 pg/mL to 1 600 pg/mL

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

For additional support contact your local Field Application Support