GYROS PROTEIN Technologies

Gyrolab® Assays

Clusterin Assay

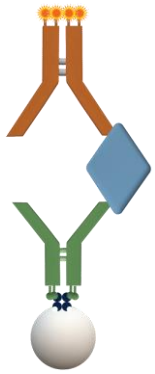
INTRODUCTION

Clusterin (APO J, SGP-2, TRPM-2, SP-40, pADHC-9, CLJ, T64, GP III, XIP8) is a 75- to 80-kDa disulfide-linked heterodimeric glycoprotein involved in the clearance of cellular debris and apoptosis. It is elevated in the kidney and urine of rats, dogs, and primates in association with the acute injury induced by ischemia/reperfusion, nephrotoxicants, and other causes of renal damage.

We have developed a three-step sandwich Gyrolab Assay to determine clusterin in rat/mouse urine 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 rabbit monoclonal antibody as a capture molecule and an Alexa Fluor® 647 labelled polyclonal goat antibody as a detection molecule.



ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 4-log standard curve (Figure 1) was generated over four runs, achieving an assay range from 0.64 ng/mL to 1 850 ng/mL (Table 1). The Limit of Detection (LOD) was 0.12 ng/mL 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 triplicates in four runs, was <20% (Table 2).

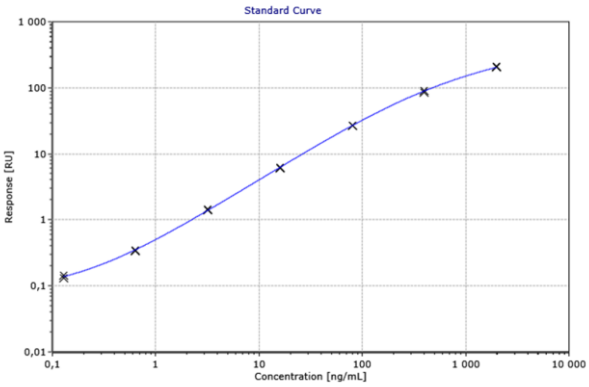


Figure 1 Standard curve in Rexxip A

Table 1 Estimated assay range, based on four runs

Assay range	LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
On plate	~ 0.12	~ 0.64	~ 1 850
In neat matrix	~ 0.36	~ 1.92	~ 5 550

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

QC	Expected Conc (ng/mL)	Average Measured Conc (ng/mL)	Inter Run CV% (n=4)	Average Intra Run CV% (n=4)	Average Total Error% (n=4)
LLOQ	0.64	0.64	11	9.5	15
LQC	7.7	7.2	4.7	4.3	11
MQC	23	22	5.2	3.3	9.1
HQC	462	476	4.3	3.3	6.3
ULOQ	1 850	1 869	7.5	7.0	11

Dilution linearity

Linearity of dilution was examined by spiking recombinant clusterin into a rat urine sample. The sample was serially diluted to obtain six data points (Table 3).

Table 3 Dilution of spiked sample.

Dilution Factor	Calculated Conc (ng/mL)	% Recovery	CV%
3	1 142	114	1.6
9	1 130	113	1.3
27	1 165	117	4.0
81	1 068	107	3.8
243	1 142	114	2.7
729	1 113	111	0.61

Parallelism

Parallelism tests could not be performed since endogenous levels of clusterin 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 xP using Gyrolab Bioaffy 1000HC CD. The assay was set up using a 3-step Gyrolab method with two wash solutions (1000 HC Assay Toolbox method). The assay buffer was Rexxip A with an MRD of 1:3. A monoclonal rabbit IgG was purchased from Invitrogen and was biotinylated according to the Gyrolab standard protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL diluted in PBS-T.

The detection antibody, labeled with Alexa Fluor 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was a polyclonal goat IgG from R&D Systems, diluted to 50 nM in Rexxip F. The assay standard used was recombinant mouse clusterin from R&D Systems diluted in Rexxip A.

Summary table

Capture	100 µg/mL biotinylated clusterin monoclonal rabbit IgG (Invitrogen, MA5-30323) in PBS-T
Detection	Alexa Fluor 647-labeled clusterin polyclonal goat IgG (R&D Systems, AF2747) 50 nM in Rexxip F
Analyte	Recombinant mouse clusterin (R&D Systems, 2747-HS) in Rexxip A
CD-type	Bioaffy 1000 HC
Method	1000 HC Assay Toolbox method
Wash buffer for needles	Wash buffer 1: PBS-T, wash buffer 2: Gyrolab wash buffer pH11
PMT-setting	1%
Expected dynamic range	Approximate 0.64 ng/mL to 1 850 ng/mL (1.92 ng/mL to 5 550 ng/mL in neat rat urine)

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.

In assays that include low affinity reagents, the Gyrolab Bioaffy 1000 HC CD can demonstrate improved performance. The 1000 HC CD is packed with porous streptavidin coated beads that have a higher capacity for biotinylated capture reagents compared to the beads in the Bioaffy 1000 CD.

For additional support contact your local Field Application Support