GYROS PROTEIN Technologies

Gyrolab® Assays

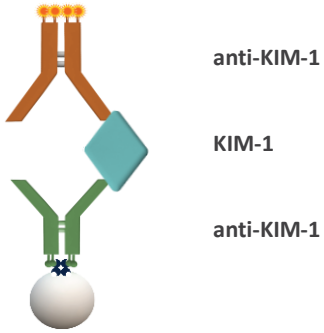
Rat KIM-1 Assay

INTRODUCTION

Kidney Injury Molecule 1 (Kim-1) is a transmembrane protein with low expression in normal kidneys. Upon injury, the expression increases and the extracellular domains of KIM-1 enters the urine. Measurements of urinary KIM-1 has emerged as a marker for acute renal tubular injury. We have developed a three-step sandwich Gyrolab toxicity biomarker assay to determine rat KIM-1 in 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 biotinylated goat anti-rat TIM-1/KIM-1/HAVCR as a capture molecule and Alexa Fluor® 647-labeled goat anti-rat TIM-1/KIM-1/HAVCR as a detection molecule.



ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 4-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 0.08 ng/mL to 300 ng/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 duplicate in three runs, was generally <20% (Table 2).

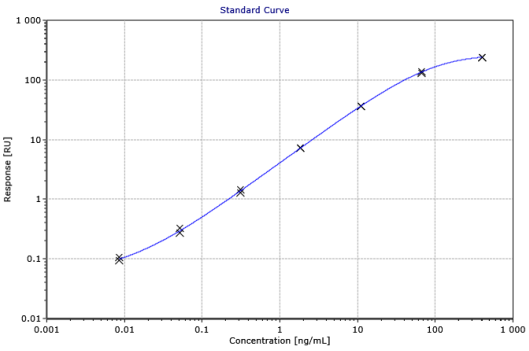


Figure 1 Standard curve in Rexxip A

Table 1 Estimated assay range, based on three runs

Assay range	LOD (ng/mL)	LLOQ (ng/mL)	ULOQ (ng/mL)
On plate	~ 0.02	~ 0.08	~ 300
In neat matrix	~ 0.04	~ 0.16	~ 600

Table 2 Accuracy and precision data of QC samples in buffer, 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	0.08	0.08	22	12.0	26
LQC	1	1	4.8	2.7	8.8
MQC	10	10	6.0	1.5	6.2
HQC	100	100	6.2	3.6	7.7
ULOQ	300	282	16	19.8	26

Parallelism

Parallelism was examined by diluting rat urine samples containing a detectable endogenous level of Kim-1. The samples were serially diluted in Rexxip A-max / Rexxip A to levels below the quantification range (Table 3). It is recommended that the end user performs parallelism assessment on urine samples with elevated endogenous levels of Kim-1.

Table 3 Parallelism

Sample	Dilution Factor	Calculated Conc (ng/mL)	CV%	Recovery % of lowest dilution
1	2	0.696	0.48	100
	4	0.702	3.5	101
	8	0.704	8.8	101
	16	< LLOQ	-	-
2	2	1.15	0.94	100
	4	1.19	3.2	103
	8	1.20	5.6	104
	16	< LLOQ	-	-

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; 1000-3W-006-A. The assay buffer was Rexxip A-max / Rexxip A with an MRD of 1:2. The 1:2 dilution was prepared in Rexxip A-max whereas the continuing dilutions were prepared in Rexxip A. Goat anti-rat TIM-1/KIM-1/HAVCR from R&D Systems (AF3689) was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 700 nM, diluted in PBST.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was goat anti-rat TIM-1/KIM-1/HAVCR from R&D Systems (AF3689), diluted to 12.5 nM in Rexxip F. The assay standard used was recombinant Rat TIM-1/KIM-1/HAVCR, from R&D Systems (3689-TM-050). The standard was prepared in Rexxip A.

Summary table

Capture	100 µg/mL biotinylated goat anti-rat TIM-1/KIM-1/HAVCR (R&D Systems, AF3689) in PBS-T
Detection	Alexa Fluor 647 labeled goat anti-rat TIM-1/KIM-1/HAVCR (R&D Systems, AF3689) 12.5 nM in Rexxip F
Analyte	Recombinant Rat TIM-1/KIM-1/HAVCR, from R&D Systems (3689-TM-050) in Rexxip A-max / Rexxip A
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 0.08 ng/mL to 300 ng/mL (0.16 ng/mL to 600 ng/mL in neat rat urine)

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