GYROS PROTEIN Technologies

Gyrolab® Assays

Human IL-8 Assay

INTRODUCTION

Interleukin 8 (IL-8) is a major mediator of the inflammatory response that is released early post-injury by a variety of tissue and blood cells. It is a chemoattractant cytokine that plays a key role in the recruitment and activation of polymorphonuclear leukocytes during an immune response in inflammatory regions. Elevated levels of IL-8 can lead to chronic inflammatory diseases, therefore its crucial to maintain a balanced IL-8 expression.

We have developed a three-step sandwich Gyrolab assay to determine IL-8 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 anti-human IL-8 monoclonal antibody as a capture molecule and an anti-human IL-8 polyclonal antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant human IL-8 (rh IL-8) was used as a standard material.

anti-IL-8 pAb

rh IL-8

anti-IL-8-mAb

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 1 pg/mL to 2 000 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).

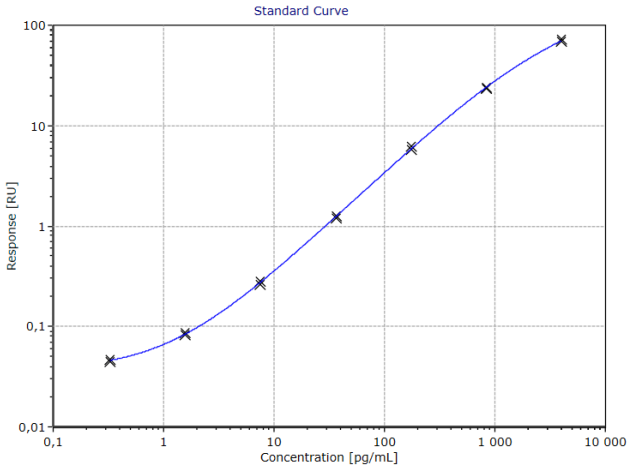

Figure 1 Standard curve in Rexxip® HN

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.2	~ 1	~ 2 000
In neat matrix	~ 0.4	~ 2	~ 4 000

Table 2 Accuracy and precision data of QC samples in Rexxip HN, 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)
QC 1	1	1.0	14	7.6	18
QC 2	3	2.8	12	8.2	15
QC 3	50	48	9.5	3.3	10
QC 4	1 600	1 525	9.6	6.5	13
QC 5	2 000	1 852	11	5.5	13

Dilution linearity

Linearity of dilution was examined by spiking recombinant human IL-8 into human serum samples. The spiked serum samples were diluted 1:2 in Rexxip HN-max and thereafter, serially diluted 1:2 in Rexxip HN (1:4-1:64) to obtain six data points (Table 3).

Table 3 Linearity of dilution. Each dilution was analyzed in triplicates.

Sample	Dilution Factor	Calculated Conc (pg/mL)	Recovery (%)	CV (%)
Human serum pool	2	185	102	7.3
	4	195	107	0.50
	8	175	96	14
	16	192	106	3.7
	32	196	108	7.1
	64	195	107	2.9
Human serum individual 1	2	170	93	4.6
	4	176	97	3.4
	8	173	95	3.8
	16	176	96	2.5
	32	170	93	4.2
	64	181	99	7.8

Parallelism

Parallelism was examined by diluting human serum samples containing a detectable endogenous level of IL-8. The samples were diluted 1:2 in Rexxip HN-max and thereafter, serially diluted 1:2 in Rexxip HN (1:4-1:32) to obtain five data points to levels below the quantification range (Table 4).

Table 4 Parallelism. Each dilution was analyzed in triplicates.

Sample	Dilution Factor	Calculated Conc (pg/mL)	CV (%)	Recovery of % of lowest dilution
Human serum individual 2	2	22	3.4	100
	4	23	5.0	106
	8	23	5.5	105
	16	22	10	100
	32	20	13	90
Human serum individual 3	2	13	3.5	100
	4	14	12	111
	8	14	0.19	107
	16	13	3.8	105
	32	12	10	97

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 method with two wash solutions (4000-3W-001-A) and a 1% PMT setting. The assay buffer was Rexxip HN. The Minimum Required Dilution (MRD) for serum samples was 1:2. Mouse monoclonal anti-human IL-8 antibody (clone 6217) from R&D Systems was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 80 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 goat polyclonal anti-human IL-8 antibody from R&D Systems (catalogue no. AF-208-NA), diluted to 5 nM in Rexxip F. The assay standard used was recombinant human IL-8, from R&D Systems (catalogue no. 208-IL-010). The standard curve was prepared in Rexxip HN.

Summary table

Capture	Mouse monoclonal anti-human IL-8 antibody (clone 6217, R&D Systems, MAB208-100), biotinylated and diluted to 80 µg/mL in PBS-T
Detection	Goat polyclonal anti-human IL-8 antibody (R&D Systems, AF-208-NA) labeled with Alexa Fluor® 647, 5 nM in Rexxip® F
Analyte	Recombinant human IL-8 (R&D Systems, 208-IL-010) in Rexxip® HN
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	1%
Expected dynamic range	Approximately 1 pg/mL to 2 000 pg/mL (2 pg/mL to 4 000 pg/mL in neat human serum, dilution 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