GYROS PROTEIN Technologies

Gyrolab® Assays

Human IL-2 Assay

INTRODUCTION

Interleukin-2 (IL-2) is a cytokine with immune-stimulatory and immune-regulatory functions that plays a crucial role in regulating immune responses and maintaining peripheral self-tolerance. IL-2 plays a central role as T-cell growth factor by stimulating the differentiation and survival of effector- and memory T-cells as well as generation and maintenance of regulatory T cells.

We have developed a three-step sandwich Gyrolab assay to quantify IL-2 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-2 monoclonal antibody as a capture molecule and an anti-human IL-2 monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant human IL-2 (rh IL-2) was used as standard material.

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 3 000 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).

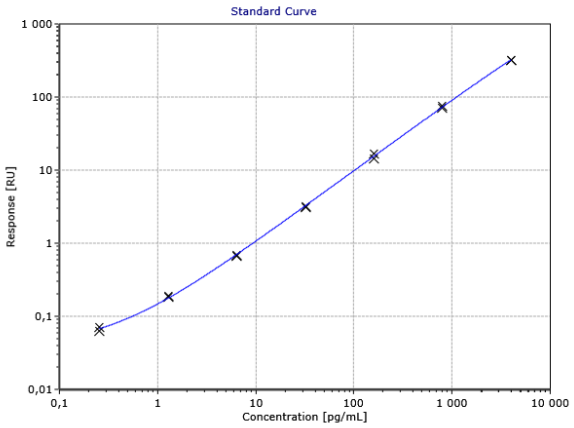

Figure 1 Standard curve in Rexxip® H

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.3	~ 1	~ 3 000
In neat matrix	~ 0.6	~ 2	~ 6 000

Table 2 Accuracy and precision data of QC samples in Rexxip H, 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)
1	1	1.1	17	13	24
2	2	2.1	11	11	18
3	5	5.0	5.9	2.4	6.7
4	10	10	10	1.8	8.7
5	100	104	1.5	3.5	7.4
6	3 000	3 001	2.4	3.3	5.0

Dilution linearity

Linearity of dilution was examined by spiking human recombinant IL-2 into human serum samples. The spiked serum samples were diluted 1:2 in Rexxip H-max and then serially diluted 1:2 in Rexxip H (1:4-1:64) to obtain six data points (Table 3). Unspiked serum measured <LLOQ.

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

Sample	Dilution Factor	Calculated Conc (pg/mL)	CV (%)	Recovery (%)
1	2	540	3.5	108
	4	480	0.67	96
	8	482	1.5	96
	16	460	4.1	92
	32	483	3.7	97
	64	490	5.4	98
2	2	523	2.4	105
	4	457	1.2	91
	8	457	3.6	91
	16	466	3.1	93
	32	455	2.9	91
	64	470	2.9	94
3	2	550	0.60	110
	4	505	1.4	101
	8	464	1.5	93
	16	464	4.7	93
	32	483	1.2	97
	64	483	3.5	97

Parallelism

Parallelism tests could not be performed since the endogenous level of human IL-2 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 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 5% PMT setting. The assay buffer was Rexxip H. The Minimum Required Dilution (MRD) for serum samples was 1:2. Mouse monoclonal anti-human IL-2 antibody (clone 5344.111) from BD Pharmingen 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 a mouse monoclonal anti-human IL-2 antibody (clone 5334) from R&D Systems, diluted to 25 nM in Rexxip F. The assay standard used was recombinant human IL-2 from R&D Systems (catalogue no. 202-IL-10). The standard curve was prepared in Rexxip H.

Summary table

Capture	Mouse monoclonal anti-human IL-2 antibody (clone 5344.111, BD Pharmingen, 555051), biotinylated and diluted to 100 µg/mL in PBS-T + 0.02% sodium azide
Detection	Mouse monoclonal anti-human IL-2 antibody (clone 5334, R&D Systems, MAB202-100) labeled with Alexa Fluor® 647, 25 nM in Rexxip® F
Analyte	Recombinant human IL-2 (R&D Systems, 202-IL-10) in Rexxip® H
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	5%
Expected dynamic range	Approximately 1 pg/mL to 3 000 pg/mL (2 pg/mL to 6 000 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