GYROS PROTEIN Technologies

Gyrolab® Assays

Human IL-12 p70 Assay

INTRODUCTION

Interleukin-12 p70 is a heterodimeric pro-inflammatory cytokine that influences the outcome of cancer, infection, and inflammatory diseases. It is produced by various cell types, such as macrophages, dendritic cells, neutrophils, and B lymphocytes in response to microbial pathogens. The secretion of IL-12 p70 leads to the activation of NK cells and differentiation of naïve CD4+ T cells into Th1 cells, thus acting as a link between the innate and adaptive immune system. Subsequently, T cells induce secretion of IFN-γ that in turn activates IL-12 p70 production, as a positive feedback mechanism.

We have developed a three-step sandwich Gyrolab assay to determine IL-12 p70 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 IL-12 p35 Monoclonal Antibody (mAb) as a capture molecule and an IL-12/IL-23 p40 mAb labeled with Alexa Fluor® 647 as a detection molecule. Recombinant human IL-12 p70 (rh IL-12 p70) was used as a standard material.

anti-IL-12 p40 mAb

rh IL-12p70

anti-IL-12 p35 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 2 pg/mL to 4 000 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).

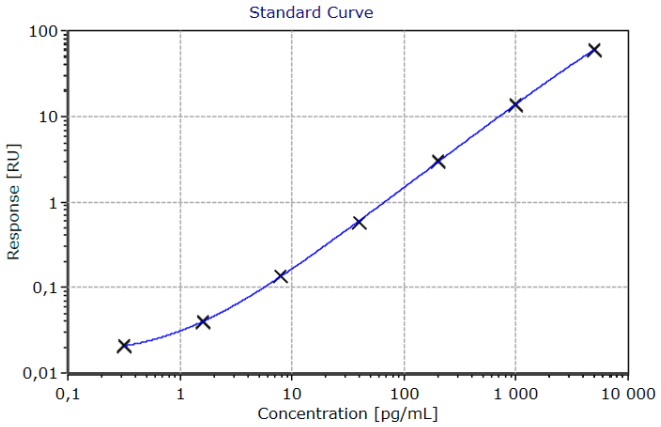

Figure 1 Standard curve in Rexxip H

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.2	~ 2	~ 4 000
In neat matrix	~ 0.4	~ 4	~ 8 000

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)
QC 1	2.0	1.9	13.9	9.1	18
QC 2	6.0	5.8	7.0	5.2	10
QC 3	100	102	7.0	4.2	8.6
QC 4	3 000	2 913	3.4	3.5	6.4
QC 5	4 000	3 827	3.9	2.3	6.7

Dilution linearity

Linearity of dilution was examined by spiking recombinant human IL-12 p70 into human serum samples. The spiked serum samples were diluted 1:2 in Rexxip H max and serially diluted 1:2 in Rexxip H 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	206	103	1.3
	4	208	104	5.8
	8	202	101	3.3
	16	204	102	6.4
	32	190	95	2.3
	64	229	115	5.9
Human serum individual 1	2	213	107	4.2
	4	213	106	1.3
	8	205	103	1.6
	16	209	105	1.7
	32	210	105	3.1
	64	213	106	0.29
Human serum individual 2	2	207	104	8.1
	4	217	109	2.2
	8	209	104	3.7
	16	205	102	0.40
	32	205	103	6.0
	64	217	109	4.7

Parallelism

Parallelism tests could not be performed since the endogenous level of human IL-12 p70 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. IL-12 p35 Monoclonal Antibody (clone B-T21) from Thermo Fisher Scientific was biotinylated according to 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 IL-12/IL-23 p40 Monoclonal Antibody (clone C8.6) from Invitrogen/Thermo Fisher Scientific, diluted to 12.5 nM in Rexxip F. The assay standard used was the recombinant human IL-12 (linked heterodimer), from R&D Systems (catalogue no 10018-IL-010). The standard was prepared in Rexxip H.

Summary table

Capture	100 µg/mL biotinylated IL-12 p35 Monoclonal Antibody (clone B-T21, Thermo Fisher Scientific) in PBS-T
Detection	Alexa Fluor 647-labeled IL-12 p40 Monoclonal Antibody (clone C8.6, Invitrogen/Thermo Fisher Scientific) 12.5 nM in Rexxip F
Analyte	Recombinant human IL-12 (linked heterodimer, R&D Systems, 10018-IL-010) 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 2 pg/mL to 4 000 pg/mL (4 pg/mL to 8 000 pg/mL in 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