GYROS PROTEIN Technologies

Gyrolab® Assays

Human IL-13 Assay

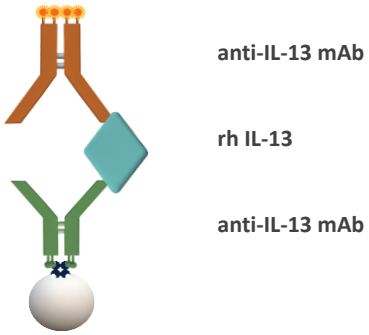
INTRODUCTION

Interleukin 13 (IL-13) is a cytokine with important immunosuppressive and anti-inflammatory activities on several effector cells of the immune system, including inhibition of pro-inflammatory cytokines. IL-13 is involved in development of type 2 T helper (T_h2) cells, IgE synthesis and eosinophil activation, and plays a key role in allergic asthma.

We have developed a three-step sandwich Gyrolab assay to determine IL-13 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-13 monoclonal antibody as a capture molecule and a different anti-human IL-13 monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant human IL-13 (rh IL-13) 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 to 1 000 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).

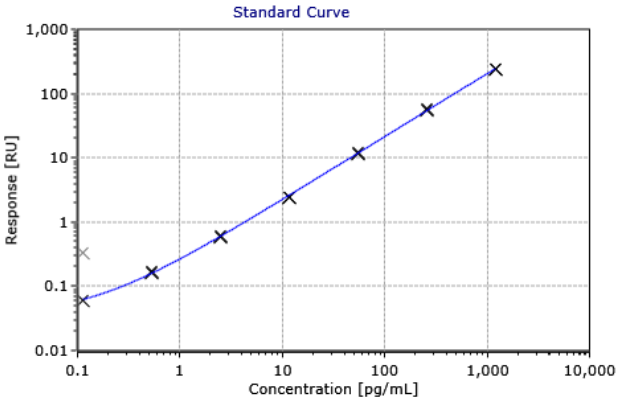


Figure 1 Standard curve in Rexxip® HN

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 0.5	~ 1	~ 1 000
In neat matrix	~ 1	~ 2	~ 2 000

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)
1	1.0	1.1	11	13	19
2	3.0	3.2	6.8	4.6	13
3	40	43	6.4	2.3	9.1
4	800	869	6.9	4.0	13
5	1 000	1 059	8.2	3.6	9.8

Dilution linearity

Linearity of dilution was examined by spiking recombinant human IL-13 into human serum samples. The spiked serum samples were diluted 1:2 in Rexxip HN-max and then 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	169	106	8.1
	4	166	104	0.4
	8	159	100	6.2
	16	159	100	3.1
	32	148	93	1.7
	64	147	92	2.2
Human serum individual 1	2	183	114	1.7
	4	172	107	0.2
	8	160	100	8.0
	16	160	100	2.0
	32	157	98	2.3
	64	159	99	3.2
Human serum individual 2	2	169	106	4.5
	4	165	103	4.3
	8	167	105	3.0
	16	166	104	2.0
	32	166	104	2.0
	64	147	92	17

Parallelism

Parallelism tests could not be performed since the endogenous level of human IL-13 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 HN. The Minimum Required Dilution (MRD) for serum samples was 1:2. Mouse monoclonal anti-human IL-13 antibody (clone 15) from Sino Biological 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 the mouse monoclonal anti-human IL-13 antibody (clone 02) from Sino Biological, diluted to 2.5 nM in Rexxip F. The assay standard used was the recombinant human IL-13, from Sino Biological (catalogue no. 10369-HNAC). The standard curve was prepared in Rexxip HN.

Summary table

Capture	Mouse monoclonal anti-human IL-13 antibody (clone 15, Sino Biological, 10369-MM15), biotinylated and diluted to 100 µg/mL in PBS-T
Detection	Mouse monoclonal anti-human IL-13 antibody (clone 02, Sino Biological, 10369-MM02T) labeled with Alexa Fluor® 647, 2.5 nM in Rexxip® F
Analyte	Recombinant human IL-13 (Sino Biological, 10369-HNAC) 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	5%
Expected dynamic range	Approximately 1 pg/mL to 1 000 pg/mL (2 pg/mL to 2 000 pg/mL in neat matrix, 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