GYROS PROTEIN Technologies

Gyrolab® Assays

Human IFNγ Assay

INTRODUCTION

Interferon gamma (IFNγ, type II interferon) is a cytokine predominantly produced by natural killer (NK) and natural killer T (NKT) cells. It exhibits both immunostimulatory and immunomodulatory effects and is critical for innate and adaptive immunity against viral infections. The homodimerized cytokine has also been shown to be elevated in cytokine release syndrome (CRS) and cytokine storm syndrome (CSS), which has been associated to therapeutic antibody therapies, CAR-T cell therapies and patients affected by infectious and noninfectious diseases, e.g. COVID-19.

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

ASSAY PERFORMANCE

Dynamic range, accuracy and precision

A robust 3-log standard curve (Figure 1) was generated over three runs, achieving an assay range from 4 pg/mL to 6 000 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 triplicates in three runs, was <20% (Table 2).

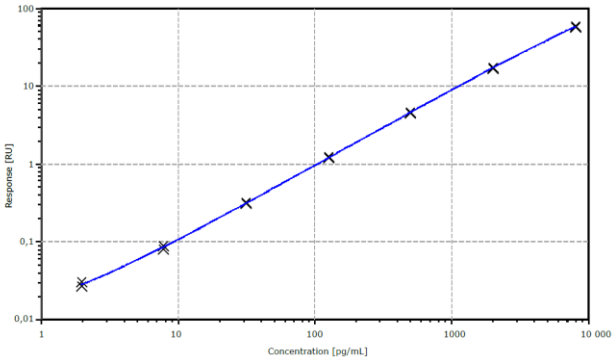

Figure 1 Standard curve in Rexxip® HN

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 1	~ 4	~ 6 000
In neat matrix	~ 2	~ 8	~ 12 000

Table 2 Accuracy and precision data of QC samples in Rexxip HN, n = number of runs

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)
4	4.1	11	9.5	18
6	6.4	2.0	4.0	12
10	11	5.4	5.8	19
20	22	7.6	4.8	16
100	94	7.8	1.7	9.3
6 000	5 928	7.2	2.1	7.0

Dilution linearity

Linearity of dilution was examined by spiking recombinant human IFNγ into two human serum samples and one human serum pool. 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)	CV (%)	Recovery (%)
1	2	479	1.6	96
	4	498	0.5	100
	8	509	0.5	102
	16	497	2.3	99
	32	465	6.4	93
	64	473	5.4	95
2	2	484	1.8	97
	4	502	4.0	100
	8	496	2.5	99
	16	482	3.1	96
	32	466	1.5	93
	64	470	2.3	94
Serum pool	2	484	5.0	97
	4	492	2.4	98
	8	490	2.3	98
	16	480	7.1	96
	32	477	3.4	95
	64	470	10.9	94

Parallelism

Parallelism tests could not be performed since the endogenous level of human IFNγ was below, or around 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 the 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. Sample preparations were performed on ice. The mouse monoclonal anti-human IFNγ, clone B133.5, from Thermo Fisher Scientific was biotinylated according the Gyrolab biotinylation protocol (Gyrolab User Guide) and used in a concentration of 100 µg/mL, diluted with PBS with 1% BSA.

The detection antibody, labeled with Alexa Fluor® 647 according to the Gyrolab standard protocol (Gyrolab User Guide), was the mouse monoclonal anti-human IFNγ, clone NIB42 from BD Pharmingen, diluted to 25 nM in Rexxip F. The assay standard used was the recombinant human IFNγ, from Thermo Fisher Scientific. The standard was prepared in Rexxip HN.

Summary table

Capture	Mouse monoclonal anti-human IFNγ antibody (clone B133.5, Thermo Fisher Scientific, cat. no. M701), biotinylated and diluted to 100 µg/mL in PBS/1% BSA
Detection	Mouse monoclonal anti-human IFNγ antibody (clone NIB42, BD Pharmingen, cat. no. 554547) labeled with Alexa Fluor® 647, 25 nM in Rexxip® F
Analyte	Recombinant human IFNγ (Thermo Fisher Scientific, cat. no. RIFNG50) 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 4 pg/mL to 6 000 pg/mL (8 pg/mL to 12 000 pg/mL in neat 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