GYROS PROTEIN Technologies

Gyrolab® Assays

Mouse IFNγ Assay

INTRODUCTION

Interferon gamma (IFNγ) is a cytokine with pleiotropic effects, mainly produced by natural killer (NK) cells and T cells. It is a pro-inflammatory cytokine that also functions as an anti-inflammatory mediator and exhibits apoptotic and antiviral effects. By promoting the development and activation of type 1 T helper (T_h1) cells, inducing immunoglobulin class switching in B cells, and upregulating antigen presentation molecules, the cytokine plays a key role in host defense.

We have developed a three-step sandwich Gyrolab assay to determine IFNγ in mouse 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-mouse IFNγ monoclonal antibody as a capture molecule and a different anti-mouse IFNγ monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant mouse IFNγ (rm IFNγ) 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 2 pg/mL to 1 000 pg/mL (Table 1). The Limits 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).

Table 1 Estimated Assay Range, based on three runs

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

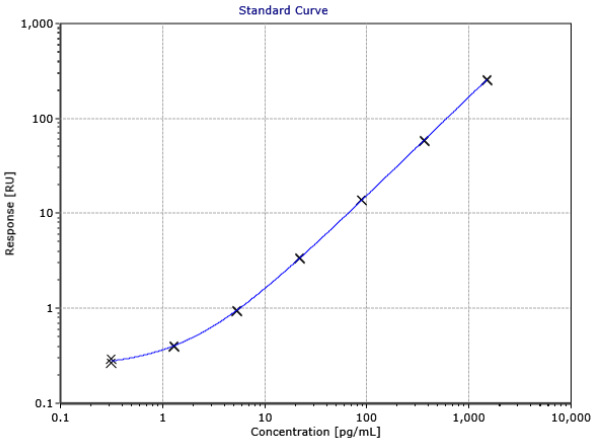

Figure 1 Standard curve in Rexxip® AN

Table 2 Accuracy and precision data of QC samples in Rexxip AN, 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	2.0	2.2	6.8	6.8	15
2	4.0	4.2	8.9	8.9	13
3	80	82	6.3	2.5	7.2
4	800	824	4.5	2.8	6.2
5	1 000	1 003	3.2	2.5	4.3

Parallelism

Parallelism was examined by diluting mouse serum samples containing a detectable endogenous level of IFNγ. The samples were diluted 1:2 in Rexxip AN-max and thereafter, serially diluted 1:2 in Rexxip AN (1:4-1:64) to levels below the quantification range (Table 3).

Table 3 Parallelism. Each dilution was analyzed in triplicates.

Sample	Dilution Factor	Calculated Conc (pg/mL)	CV (%)	Recovery % of lowest dilution
Mouse serum pool BALB/c	2	32	5.0	100
	4	30	8.0	94
	8	26	9.0	82
	16	<LLOQ	-	-
	32	<LLOQ	-	-
	64	<LLOQ	-	-
Mouse serum individual 1 BALB/c	2	75	3.4	100
	4	73	1.1	96
	8	71	7.5	94
	16	70	4.1	93
	32	73	9.8	96
	64	<LLOQ	-	-
Mouse serum individual 2 BALB/c	2	81	5.5	100
	4	78	5.1	96
	8	80	4.8	99
	16	75	8.8	92
	32	75	1.1	92
	64	<LLOQ	-	-

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 AN. The minimum required dilution (MRD) for serum samples was 1:2. Rat monoclonal anti-mouse IFNγ antibody (clone AN-18) from Biolegend 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 rat monoclonal anti-mouse IFNγ antibody (clone R4-6A2) from Biolegend, diluted to 10 nM in Rexxip F. The assay standard used was the recombinant mouse IFNγ, from R&D Systems (catalogue no. 485-MI-100). The standard curve was prepared in Rexxip AN.

Summary table

Capture	Rat monoclonal anti-mouse IFNγ antibody (clone AN-18, Biolegend, 517902), biotinylated and diluted to 100 µg/mL in PBS-T
Detection	Rat monoclonal anti-mouse IFNγ antibody (clone R4-6A2, Biolegend, 505702) labeled with Alexa Fluor® 647, 10 nM in Rexxip® F
Analyte	Recombinant mouse IFNγ (R&D Systems, 485-MI-100) in Rexxip® AN
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 1 000 pg/mL (4 pg/mL to 2 000 pg/mL in neat matrix, diluted 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