GYROS PROTEIN Technologies

Gyrolab® Assays

Human TNF-α Assay

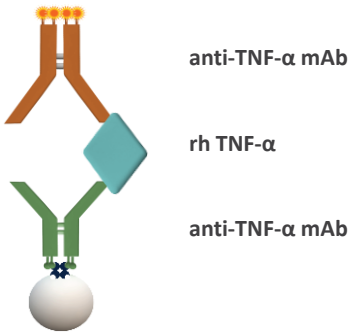
INTRODUCTION

Tumor necrosis factor alpha (TNF-α) is an inflammatory cytokine, mainly produced by macrophages. Binding of TNF-α to its receptors activates several signaling pathways, generating biological effects including activation of cells, proinflammatory cytokine production, inhibition of regulatory T-cells, and induction of apoptosis. TNF-α plays an essential role in the cytokine storm and is involved in cytokine release syndrome (CRS), which has been associated with infectious diseases like COVID-19.

We have developed a three-step sandwich Gyrolab assay to quantify TNF-α 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 TNF-α monoclonal antibody as a capture molecule and an anti-human TNF-α monoclonal antibody labeled with Alexa Fluor® 647 as a detection molecule. Recombinant human TNF-α (rh TNF-α) 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 4 pg/mL to 20 000 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 <25% (Table 2).

Table 1 Estimated Assay Range, based on three runs

Assay range	LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
On plate	~ 2.5	~ 4	~ 20 000
In neat matrix	~ 5	~ 8	~ 40 000

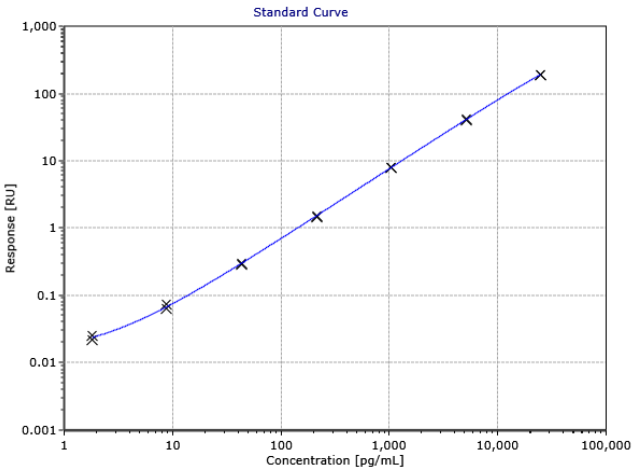


Figure 1 Standard curve in Rexxip® H

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)
LLOQ	4	3.8	22	23	33
LQC	8	7.4	8.5	5.4	14
MQC	500	451	10	2.3	13
HQC	15 000	13 725	4.6	2.0	10
ULOQ	20 000	17 857	5.8	3.7	14

Dilution linearity

Linearity of dilution was examined by spiking human recombinant TNF-α 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 triplicate.

Sample	Dilution Factor	Calculated Conc (pg/mL)	Recovery (%)	CV (%)
Human serum pool	2	661	88	1.1
	4	690	92	0.84
	8	701	93	2.4
	16	710	95	2.7
	32	759	101	7.3
	64	707	94	2.0
Human serum individual 1	2	672	90	1.1
	4	673	90	1.9
	8	702	94	3.7
	16	706	94	1.4
	32	696	93	2.1
	64	724	97	1.5
Human serum individual 2	2	656	87	0.37
	4	634	85	1.2
	8	650	87	4.9
	16	685	91	2.5
	32	660	88	2.6
	64	670	89	5.8

Parallelism

Parallelism tests could not be performed since endogenous levels of human TNF-α were below the quantification range. It is recommended that the end user performs parallelism assessment 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 1% PMT setting. The assay buffer was Rexxip H. The Minimum Required Dilution (MRD) for serum samples was 1:2. Biotinylated mouse monoclonal anti-human TNF-α antibody from Thermo Fisher Scientific (clone 68B 3C5) was 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 TNF-α antibody from BD Pharmingen (clone MAb1), diluted to 12.5 nM in Rexxip F. The assay standard used was the recombinant human TNF-α, from Prospec (catalogue no. CYT-223). The standard curve was prepared in Rexxip H.

Summary table

Capture	Biotinylated mouse monoclonal anti-human TNF-α antibody (clone 68B 3C5, Thermo Fisher Scientific, AHC3419), diluted to 100 µg/mL in PBS-T
Detection	Mouse monoclonal anti-human TNF-α antibody (clone MAb1, BD Pharmingen, 551220) labeled with Alexa Fluor® 647, 12.5 nM in Rexxip® F
Analyte	Recombinant human TNF-α (Prospec, CYT-223) 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	1%
Expected dynamic range	Approximately 4 pg/mL to 20 000 pg/mL (8 pg/mL to 40 000 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