Gyrolab® Human Cytokine Kit Reagents

For the detection of selected cytokines in human serum

Product Information Sheet D0043436/A

- **Automated assays – minimal manual operations**
- **Robust, reproducible, and reliable data – suitable for use throughout development in regulated studies**
- **High sensitivity & broad dynamic range – cover the wide range of cytokine levels that may be seen in disease states or pharmacodynamic studies**
- **High throughput – 96 data points in 90 minutes, up to 960 data points in a working day**

Introduction

Protein biomarkers are valuable at many stages of the drug development process, from understanding diseases and pathways, to identifying early diagnostic markers of disease, finding novel drug targets, and improving the designs of clinical trials. Given their importance, sensitive, precise and robust fit-for-purpose biomarker immunoassay methods are needed to enable biomarker quantification and support preclinical and clinical biotherapeutic development.

Cytokines, including interleukins, interferons, tumor necrosis factors, and chemokines, have a variety of pro- and anti-inflammatory effects that are important in health and disease states that include infection, inflammation, trauma, sepsis, and cancer. Gyros Protein Technologies has developed a range of kit reagents to meet the need to measure reliably cytokine biomarkers in human serum samples (Table 1). The kit reagents can also be used to measure cytokine levels in cell culture supernatants.

Gyrolab Human Cytokine Kit Reagents contain ready-to-use reagents, including standard material and buffers for one CD run (96 data points). While the kit reagents have been optimized to be used in combination with Gyrolab® Bioaffy™ 4000 CD, other Gyrolab CD types can be used to customize the technical performance to meet the needs of the application.

Gyrolab Human Cytokine Kit Reagents are for research use only and are not intended for diagnostic use.

Gyrolab Human Cytokine Kit Reagents enable the efficient measurement of cytokine biomarkers in human serum:

- Automation generates 96 data points within 90 minutes without manual intervention
- Broad dynamic range minimizes dilutions needed, thus, simplifying spike recovery and dilution linearity experiments, reducing variability due to errors and speeding up workflows
- Data robustness and reproducibility enables smooth transfer to other labs, sites or contract research organizations and easy validation for regulatory submissions
- Plug-and-play products that offer convenience and expedited analytical solution by removing the need for assay development

GYROS PROTEIN Technologies

Assay principle

The range of Gyrolab® Human Cytokine Kit Reagents have been developed to quantify cytokines using a sandwich immunoassay run on a Gyrolab CD of the user's choice (Figure 1). The biotinylated anti-cytokine antibody is automatically introduced into a microstructure in the Gyrolab Bioaffy CD and captured on streptavidin-coated beads in the flow-through affinity column. Samples containing cytokines are introduced into the microstructures and captured by the immobilized anti-cytokine antibody. Bound cytokine is then detected using an anti-cytokine antibody labeled with Alexa Fluor®647. Results are evaluated using Gyrolab Evaluator or exported to a LIMS. All Gyrolab software programs are designed for 21 CFR part 11-compliance, ensuring that assays can be developed and transferred in regulated environments.

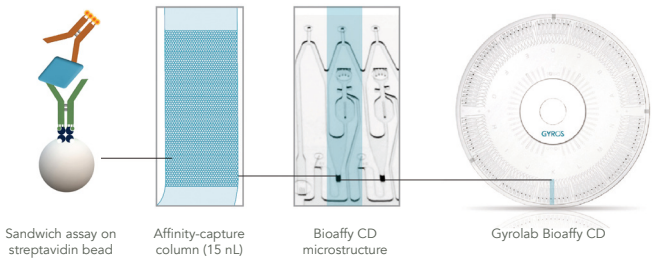

Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy CD.

Assay performance data

Sensitivity and assay range

LOD (Limit of Detection) was determined as two standard deviations (SD) above the blank. Lower Limit of Quantitation (LLOQ) and Upper Limit of Quantitation (ULOQ) were determined by analyzing QC samples from six runs by two operators over two days in the lower and upper regions of the assay's analytical range, respectively. The lowest (LLOQ) and highest (ULOQ) concentration with CV<25%, Bias <25% and Total Error (%Relative Error + %Coefficient of Variation) <40% were assigned as LLOQ and ULOQ, respectively.

Precision and accuracy

Intra- and inter-run precision were determined for four QC samples with different concentrations of recombinant human cytokine analyzed in triplicate in six runs by two operators over two days.

Dilution linearity and spike recovery

Recombinant human cytokine was spiked into three serum samples from human and cynomolgus monkey (not applicable for IL-10) with low endogenous levels. The samples were analyzed both unspiked and spiked with recombinant cytokine and analyzed neat (undiluted) and diluted in Biomarker Sample Dilution Buffer 1. The endogenous levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to the measured concentration of the spike solution. Note that unknown samples can be analyzed for dilution linearity and spike recovery 1:2 or neat and should be tested in the matrix relevant for the study.

Parallelism

Cell supernatant from stimulated human peripheral blood mononuclear cells (PBMCs) was used to study parallelism of endogenous cytokine. The cells were stimulated with phytohemagglutinin (PHA) or lipopolysaccharide (LPS) to secrete cytokines. The supernatant was diluted with Biomarker Sample Dilution Buffer 1. The parallelism of the endogenous cytokine was compared to the recombinant cytokine used in the standard curve.

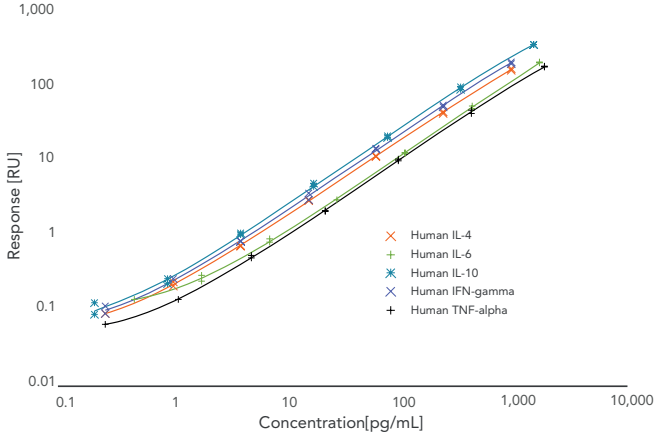

Figure 2. Standard curves for all five cytokines run sequentially in a 5CD Gyroplex format.

Cytokine: IL-4

Standard curve and assay range

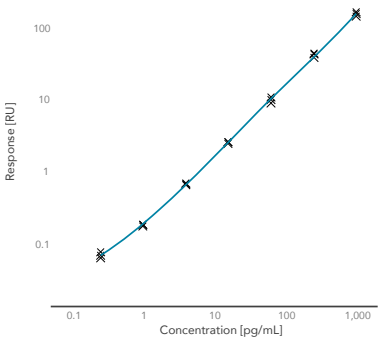

Figure 3. A typical example of recombinant human IL-4 standard curve from a Gyrolab run. Each standard sample was analyzed in triplicate.

Table 2. Assay range of Gyrolab Human IL-4 Kit Reagents

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 0.5	~ 0.8	~800

Precision and accuracy

Table 3. Intra- and inter-run precision data for QC samples covering the working range for the IL-4 assay.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	800	710	1.8 – 15.3	10.5
MQC	50.0	43.5	3.8 – 12.7	10.1
LQC	2.00	1.80	5.7 – 15.8	10.3
LLOQ	0.80	0.788	1.1 – 21.9	14.8

Dilution linearity and spike recovery

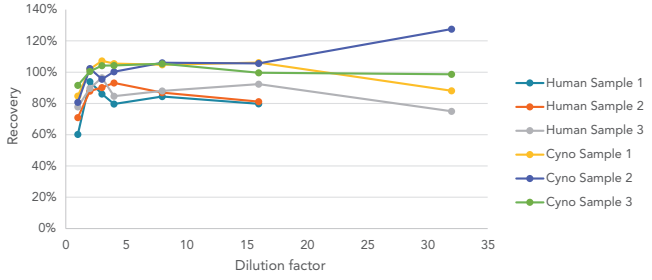

Figure 4. Dilution linearity and recovery of spiked serum samples. Serum samples were spiked with recombinant IL-4. The endogenous levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to measured concentration of the spike solution.

Parallelism

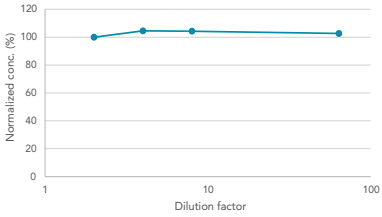

Figure 5. Back-calculated concentrations of endogenous IL-4 in cell supernatant, normalized to the concentration at lowest dilution.

Cytokine: IL-6

Standard curve and assay range

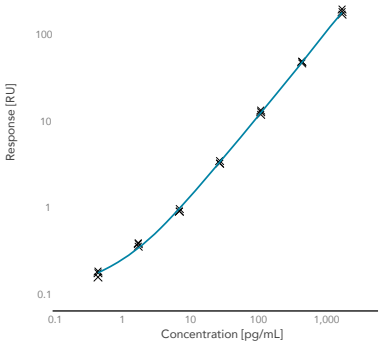

Figure 6. A typical example of recombinant human IL-6 standard curve from a Gyrolab run. Each standard sample was analyzed in triplicate.

Table 4. Assay range of Gyrolab Human IL-6 Kit Reagents

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 1.5	~ 2.0	~1400

Precision and accuracy

Table 5. Intra- and inter-run precision data for QC samples covering the working range for the IL-6 assay.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	1400	1559	1.8 – 7.0	5.1
MQC	70.0	76.8	1.1 – 8.5	5.8
LQC	4.00	4.26	3.2 – 17.6	16.5
LLOQ	2.00	2.20	7.2 – 20.5	18.9

Dilution linearity and spike recovery

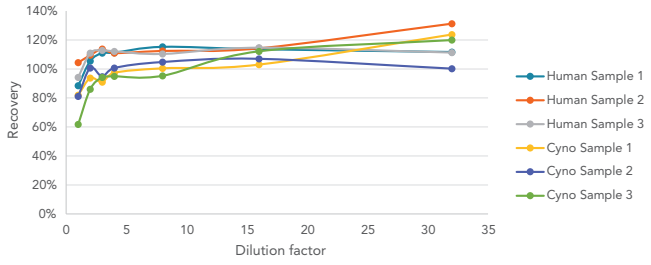

Figure 7. Dilution linearity and recovery of spiked serum samples. Serum samples were spiked with recombinant IL-6. The endogenous levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to measured concentration of the spike solution.

Parallelism

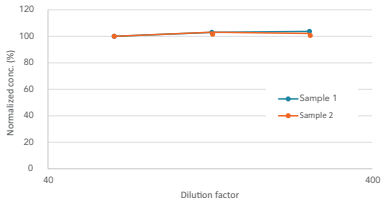

Figure 8. Back-calculated concentrations of endogenous IL-6 in cell supernatants, normalized to the concentration at lowest dilution.

Cytokine: IL-10

Standard curve and assay range

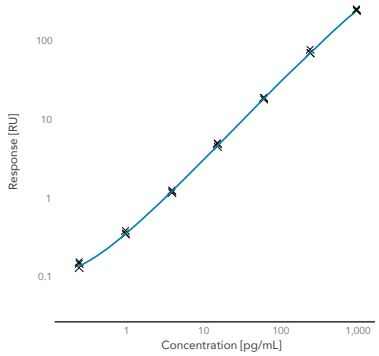

Figure 9. A typical example of recombinant human IL-10 standard curve from a Gyrolab run. Each standard sample was analyzed in triplicate.

Table 6. Assay range of Gyrolab Human IL-10 Kit Reagents

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 0.3	~ 0.75	~1200

Precision and accuracy

Table 7. Intra- and inter-run precision data for QC samples covering the working range for the IL-10 assay.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	1200	1210	1.3 – 8.4	3.9
MQC	50	49.7	0.4 – 5.1	4.7
LQC	2.25	2.25	2.4 – 13	14
LLOQ	0.75	0.74	2.5 – 17	16

Dilution linearity and spike recovery

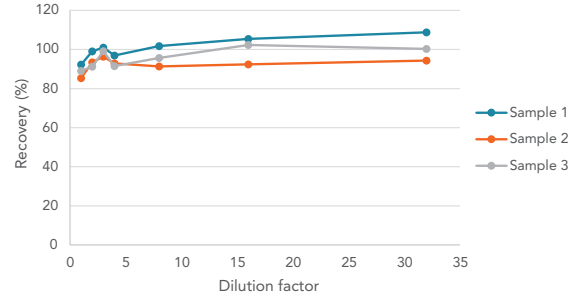

Figure 10. Dilution linearity and recovery of spiked serum samples. Serum samples were spiked with recombinant IL-10. The endogenous levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to measured concentration of the spike solution.

Parallelism

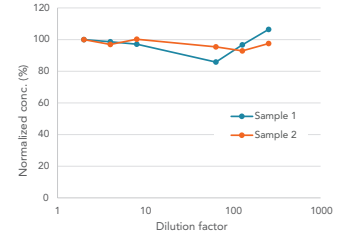

Figure 11. Back-calculated concentrations of endogenous IL-10 in cell supernatants, normalized to the concentration at lowest dilution.

Cytokine: IFN-gamma

Standard curve and assay range

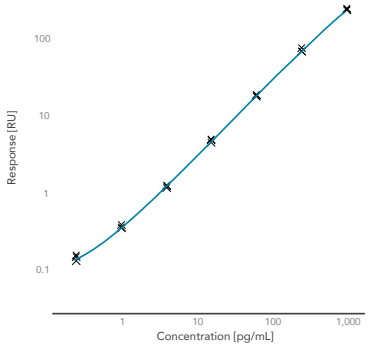

Figure 12. A typical example of recombinant human IFN-gamma standard curve from a Gyrolab run. Each standard sample was analyzed in triplicate.

Table 8. Assay range of Gyrolab Human IFN-gamma Kit Reagents.

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 0.4	~ 1	~800

Precision and accuracy

Table 9. Intra- and inter-run precision data for QC samples covering the working range for the IFN-gamma assay.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	800	726	3.6 – 5.4	4.7
MQC	30.0	28.8	3.5 – 10.0	6.0
LQC	3.00	2.77	1.2 – 10.4	7.0
LLOQ	1.00	0.931	3.3 – 7.8	9.8

Dilution linearity and spike recovery

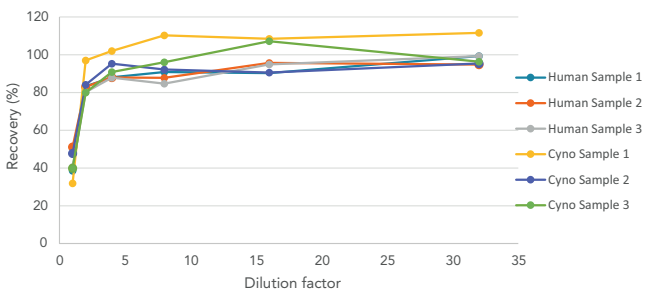

Figure 13. Dilution linearity and recovery of spiked serum samples. Serum samples were spiked with recombinant IFN-gamma. The endogenous levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to measured concentration of the spike solution.

Parallelism

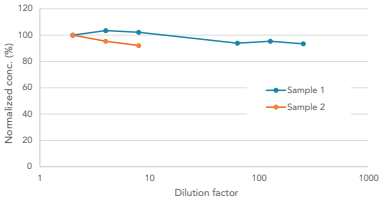

Figure 14. Back-calculated concentrations of endogenous IFN-gamma in cell supernatants, normalized to the concentration at lowest dilution.

Cytokine: TNF-alpha

Standard curve and assay range

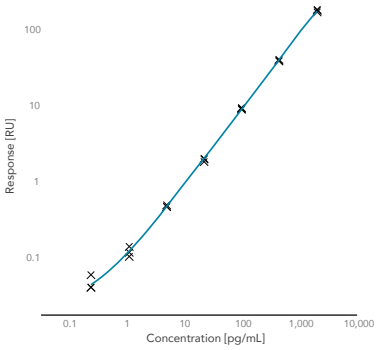

Figure 15. A typical example of recombinant human TNF-alpha standard curve from a Gyrolab run. Each standard sample was analyzed in triplicate.

Table 10. Assay range of Gyrolab Human TNF-alpha Kit Reagents.

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 0.7	~ 1.0	~1500

Precision and accuracy

Table 11. Intra- and inter-run precision data for QC samples covering the working range for the TFN-alpha assay.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	1500	1537	2.2 – 8.6	6.3
MQC	40.0	42.5	0.1 – 6.2	5.0
LQC	2.00	2.20	1.1 – 12.4	19.5
LLOQ	1.00	1.02	2.2 – 15.5	17.1

Dilution linearity and spike recovery

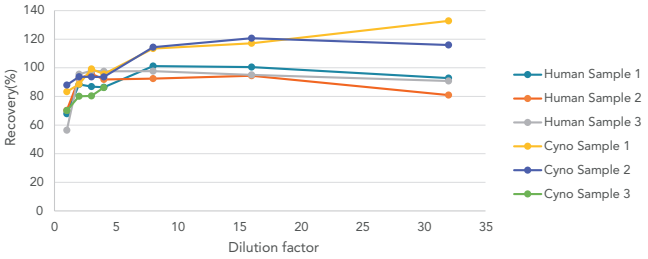

Figure 16. Dilution linearity and recovery of spiked serum samples. Serum samples were spiked with recombinant TNF-alpha. The endogenous levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to measured concentration of the spike solution.

Parallelism

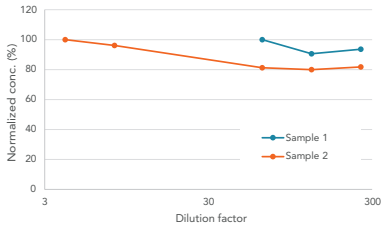

Figure 17. Back-calculated concentrations of endogenous TNF-alpha in cell supernatants, normalized to the concentration at lowest dilution.

Ordering Information

Product Number	Product name	Description
P0020822	Gyrolab Human IL-4 Kit Reagents	Includes capture and detect reagents, standard, sample dilution buffer and wash buffer required to generate 96 datapoints (1 CD)
P0020827	Gyrolab Human IL-4 Standard	Standard for spiking experiments, 16 000 pg/mL, 200 µL
P0020823	Gyrolab Human IL-6 Kit Reagents	Includes capture and detect reagents, standard, sample dilution buffer and wash buffer required to generate 96 datapoints (1 CD)
P0020828	Gyrolab Human IL-6 Standard	Standard for spiking experiments, 28 800 pg/mL, 200 µL
P0020821	Gyrolab Human IL-10 Kit Reagents	Includes capture and detect reagents, standard, sample dilution buffer and wash buffer required to generate 96 datapoints (1 CD)
P0020826	Gyrolab Human IL-10 Standard	Standard for spiking experiments, 25 600 pg/mL, 200 µL
P0020824	Gyrolab Human IFN-gamma Kit Reagents	Includes capture and detect reagents, standard, sample dilution buffer and wash buffer required to generate 96 datapoints (1 CD)
P0020829	Gyrolab Human IFN-gamma Standard	Standard for spiking experiments, 16 000 pg/mL, 200 µL
P0020825	Gyrolab Human TNF-alpha Kit Reagents	Includes capture and detect reagents, standard, sample dilution buffer and wash buffer required to generate 96 datapoints (1 CD)
P0020830	Gyrolab Human TNF-alpha Standard	Standard for spiking experiments, 1 µg/mL, 200 µL
P0020831	Gyrolab Biomarker Sample Dilution Buffer 1	Extra sample dilution buffer, 25 mL
P0020705	Gyrolab Bioaffy 4000 CD	4000 nL, 96 datapoints. For applications requiring augmented sensitivity, e.g., biomarkers.

Gyrolab Human Cytokine Kit Reagents content

Description	Gyrolab Human IL-10 Kit Reagents	Gyrolab Human IL-4 Kit Reagents	Gyrolab Human IL-6 Kit Reagents	Gyrolab Human IFN- gamma Kit Reagents	Gyrolab Human TNF- alpha Kit Reagents
Contents	P0020821	P0020822	P0020823	P0020824	P0020825
Capture Reagent	Biotinylated Anti-human IL-10 antibody, ready-to-use solution, 60 µL	Biotinylated Anti-human IL-4 antibody, ready-to-use solution, 60 µL	Biotinylated Anti-human IL-6 antibody, ready-to-use solution, 60 µL	Biotinylated Anti-human IFN-gamma antibody, ready-to-use solution, 60 µL	Biotinylated Anti-human TNF-alpha antibody, ready-to-use solution, 60 µL
Detection Reagent	Alexa Fluor™ 647 labeled Anti-human IL-10 antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-human IL-4 antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-human IL-6 antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-human IFN-gamma antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-human TNF-alpha antibody, ready-to-use solution, 60 µL
Standard	Stock solution of recombinant human IL-10, 50 µL	Stock solution of recombinant human IL-4, 50 µL	Stock solution of recombinant human IL-6, 50 µL	Stock solution of recombinant human IFN-gamma, 50 µL	Stock solution of recombinant human TNF-alpha, 50 µL
Biomarker Wash Buffer 1	1500 µL	1500 µL	1500 µL	1500 µL	1500 µL
Biomarker Sample Dilution Buffer 1	1750 µL	1750 µL	1750 µL	1750 µL	1750 µL

Storage conditions:

Refrigerate at +4°C to +8°C.

Shelf life:

See IFUs.

D0043436/A