

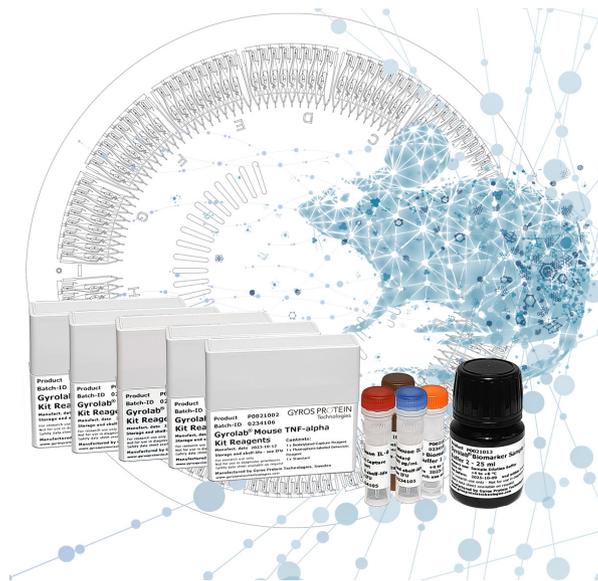
# Gyrolab<sup>®</sup> Mouse Cytokine Kits

For the detection of selected cytokines including IL-4, IL-6, IL-10, TNF- $\alpha$ , and IFN- $\gamma$  in mouse serum

Product Information Sheet

D0047494/A

- Automated workflows – reduced manual operations and variability
- Robust, reproducible, and reliable data – suitable for use throughout development in pre clinical analysis
- 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
- Mouse Gyroplex<sup>®</sup> allows for analysis of multiple Biomarkers with zero cross talk – Samples analysed sequentially with minimal manual operations



## 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, to 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 kits to meet the need to measure cytokine biomarkers in mouse serum samples.

Gyrolab Mouse Biomarker Kits contain ready-to-use reagents, including standard material and buffers for one CD run (96 data points). While the kits have been optimized to be used in combination with Gyrolab Bioaffy<sup>™</sup> 4000 CD, other Gyrolab CD types can be used to customize the technical assay performance to meet the needs of the application.

Gyrolab Mouse Biomarker Kits are for research use only and are not intended for diagnostic use.

### Gyrolab Biomarker Kits enable the efficient measurement of cytokine biomarkers in mouse serum in single-analyte assays:

- 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
- Data robustness and reproducibility enables smooth transfer to other labs, sites or contract research organizations and easy validation for regulatory submissions
- Short turnaround time and reduced manual intervention accelerates data-driven decision making and frees up operator time for more important tasks
- Low sample volume requirements of 12  $\mu$ L for duplicate analysis allow serial mouse serum sampling to minimize animal use

## Assay principle

Gyrolab® Mouse Biomarker Kits have been developed to quantify cytokines using a sandwich immunoassay run on a Gyrolab Bioaffy 4000 CD (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.

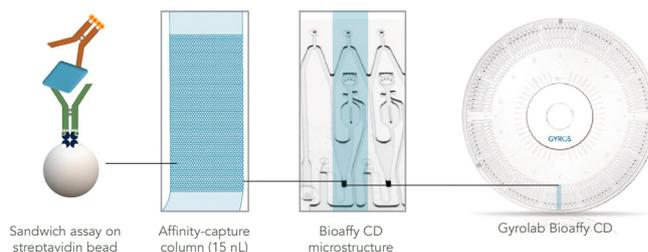


Figure 1. Sandwich immunoassay format on a Gyrolab Bioaffy CD.

## 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 six QC samples 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 mouse cytokine analyzed in triplicate in six runs by two operators over two days.

### Dilution linearity and spike recovery

Recombinant mouse cytokine was spiked into three serum samples from mice with low endogenous levels. The samples were analyzed both unspiked and spiked with recombinant cytokine and analyzed neat (undiluted) and diluted in Gyrolab Biomarker Sample Dilution Buffer 2. 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 have been analyzed for dilution linearity and spike recovery 1:2 but should be tested by the user in the matrix relevant for the study.

### Parallelism

Serum samples from mice with quantifiable endogenous levels were used to study the parallelism of endogenous cytokines. Mouse serum was diluted with Biomarker Sample Dilution Buffer 2. The parallelism of the endogenous cytokine was assessed against the recombinant cytokine used to prepare the standard curve.

### Gyroplex®

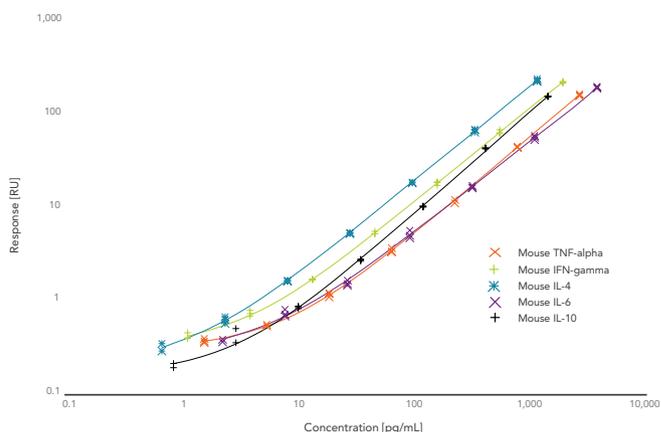
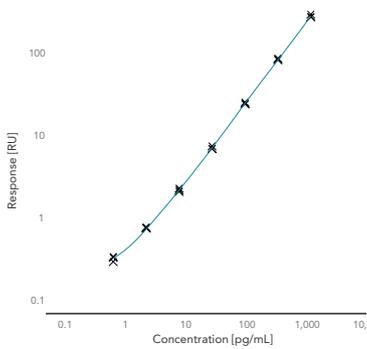


Figure 2. Standard curves for all five cytokines run sequentially in a 5CD Gyroplex format.

## Gyrolab Mouse IL-4 Kit

### Standard curve and assay range



**Figure 3.** A typical example of recombinant mouse IL-4 standard curve in Gyrolab Biomarker Sample Dilution Buffer 2 from a Gyrolab run using the Gyrolab Mouse IL-4 Kit. Each standard sample was analyzed in triplicate.

**Table 1.** Assay range of Gyrolab Mouse IL-4 Kit.

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 2	~ 3.0	~900

### Calibration

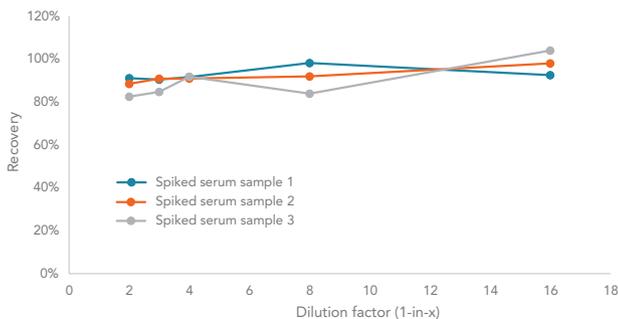
The NIBSC reference standard material 91/656 was evaluated with this kit product, with 1 ng equalling 22 IU.

### Precision and accuracy

**Table 2.** Intra- and inter-run precision data for QC samples covering the working range for the Gyrolab Mouse IL-4 Kit.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	900	959	2.6 – 16.7	11.7
MQC	30.0	33.6	2.5 – 12.7	9.6
LQC	6.00	6.93	1.7 – 11.7	6.1
LLOQ	3.00	3.51	2.2 – 8.8	7.3

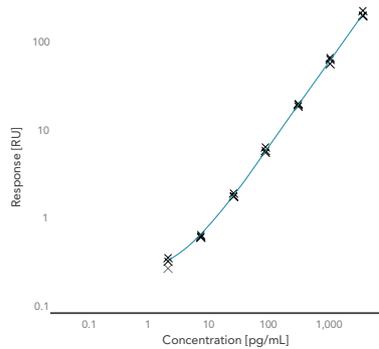
### Dilution linearity and spike recovery



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

## Gyrolab Mouse IL-6 Kit

### Standard curve and assay range



**Figure 5.** A typical example of recombinant mouse IL-6 standard curve in Gyrolab Biomarker Sample Dilution Buffer 2 from a Gyrolab run using the Gyrolab Mouse IL-6 Kit. Each standard sample was analyzed in triplicate.

**Table 3.** Assay range of Gyrolab Mouse IL-6 Kit.

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 4	~ 8.0	~3000

### Calibration

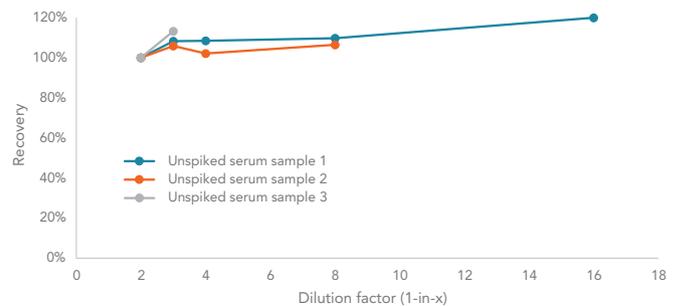
The NIBSC reference standard material 93/730 was evaluated with this kit product, with 1 ng equalling 1198 IU.

### Precision and accuracy

**Table 4.** Intra- and inter-run precision data for QC samples covering the working range for the Gyrolab Mouse IL-6 Kit.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	3000	3230	1.9 – 8.8	5.7
MQC	100	99.4	2.4 – 10.7	7.8
LQC	16.0	16.4	0.8 – 8.2	7.0
LLOQ	8.00	9.02	4.2 – 22.3	13.0

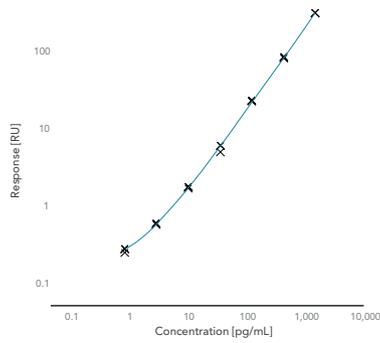
### Parallelism



**Figure 6.** Recovery after dilution of endogenous IL-6 in mouse serum samples.

## Gyrolab Mouse IL-10 Kit

### Standard curve and assay range



**Figure 7.** A typical example of recombinant mouse IL-10 standard curve in Gyrolab Biomarker Sample Dilution Buffer 2 from a Gyrolab run using the Gyrolab Mouse IL-10 Kit. Each standard sample was analyzed in triplicate.

**Table 5.** Assay range of Gyrolab Mouse IL-10 Kit.

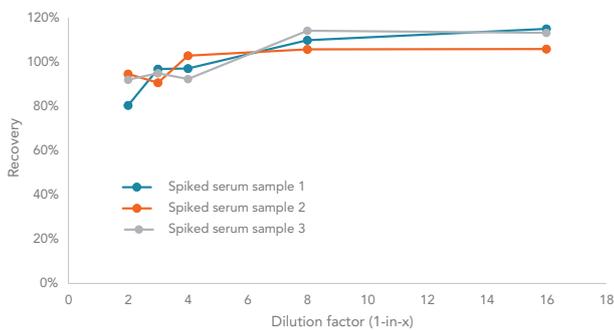
LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
< 2	~4.0	~1200

### Precision and accuracy

**Table 6.** Intra- and inter-run precision data for QC samples covering the working range for the Gyrolab Mouse IL-10 Kit.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	1200	1241	0.3 – 7.2	4.5
MQC	40.0	40.9	2.8 – 19.3	5.2
LQC	8.00	8.86	1.9 – 10.3	6.6
LLOQ	4.00	4.47	1.5 – 12.7	9.7

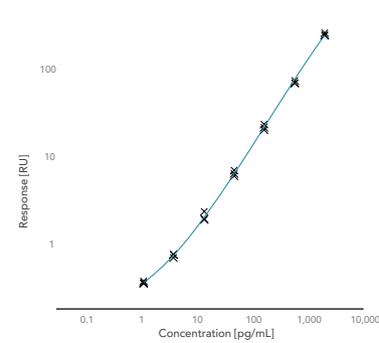
### Dilution linearity and spike recovery



**Figure 8.** Recovery of serum samples spiked with recombinant IL-10. The endogenous IL-10 levels were subtracted from the back-calculated concentrations. Recovery was calculated compared to measured concentration of the spike solution.

## Gyrolab Mouse IFN-gamma Kit

### Standard curve and assay range



**Figure 9.** A typical example of recombinant mouse IFN-gamma standard curve in Gyrolab Biomarker Sample Dilution Buffer 2 from a Gyrolab run using the Gyrolab Mouse IFN-gamma Kit. Each standard sample was analyzed in triplicate.

**Table 7.** Assay range of Gyrolab Mouse IFN-gamma Kit.

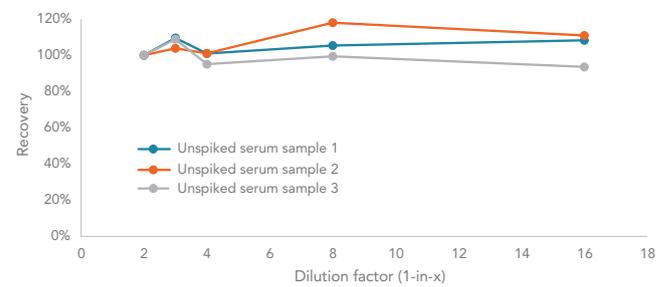
LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
<2	~4.0	~1600

### Precision and accuracy

**Table 8.** Intra- and inter-run precision data for QC samples covering the working range for the Gyrolab IFN-gamma Kit.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	1600	1687	2.4 – 7.3	6.0
MQC	50.0	49.0	2.8 – 8.4	6.6
LQC	8.00	8.05	2.5 – 12.7	8.7
LLOQ	4.00	4.25	2.5 – 16.8	14.4

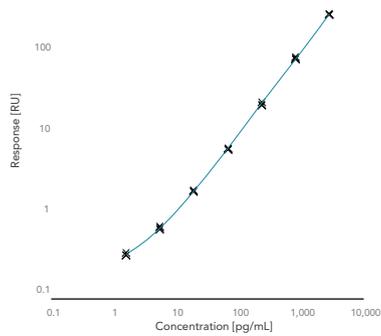
### Parallelism



**Figure 10.** Recovery after dilution of endogenous IFN-gamma in mouse serum samples.

## Gyrolab Mouse TNF-alpha Kit

### Standard curve and assay range



**Figure 11.** A typical example of recombinant mouse TNF-alpha standard curve in Gyrolab Biomarker Sample Dilution Buffer 2 from a Gyrolab run using the Gyrolab Mouse TNF-alpha Kit. Each standard sample was analyzed in triplicate.

**Table 9.** Assay range of Gyrolab Mouse TNF-alpha Kit.

LOD (pg/mL)	LLOQ (pg/mL)	ULOQ (pg/mL)
<3	~5.0	~2200

### Calibration

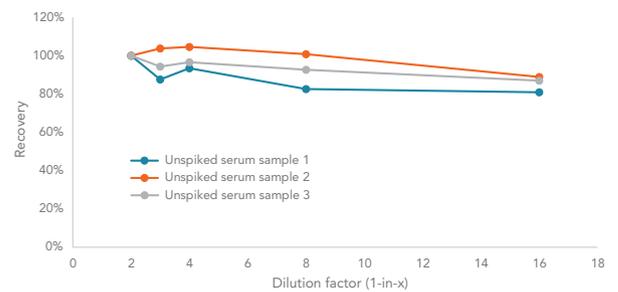
The NIBSC reference standard material 88/532 was evaluated with this kit product, with 1 ng equaling 615 IU.

### Precision and accuracy

**Table 11.** Intra- and inter-run precision data for QC samples covering the working range for the Gyrolab TNF-alpha Kit.

Sample	Nominal conc. (pg/mL)	Average measured conc. (pg/mL)	Intra-run CV (%)	Inter-run CV (%)
ULOQ/HQC	2200	2054	1.8 – 6.8	4.9
MQC	70.0	65.1	2.0 – 12.3	6.0
LQC	15.0	13.9	5.8 – 12.7	9.9
LLOQ	5.00	5.03	4.7 – 16.3	10.4

### Parallelism



**Figure 12.** Recovery after dilution of endogenous TNF-alpha in mouse serum samples.

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## Ordering Information

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Product Number	Product name	Description
P0021004	Gyrolab Mouse IL-4 Kit	The kit includes 1 CD and all reagents and consumables to generate 96 datapoints
P0021009	Gyrolab Mouse IL-4 Standard	Standard for spiking experiments, 48 000 pg/mL, 100 µL
P0021005	Gyrolab Mouse IL-6 Kit	The kit includes 1 CD and all reagents and consumables to generate 96 datapoints
P0021010	Gyrolab Mouse IL-6 Standard	Standard for spiking experiments, 120 000 pg/mL, 100 µL
P0021003	Gyrolab Mouse IL-10 Kit	The kit includes 1 CD and all reagents and consumables to generate 96 datapoints
P0021008	Gyrolab Mouse IL-10 Standard	Standard for spiking experiments, 1 µg/mL, 100 µL
P0021006	Gyrolab Mouse IFN-gamma Kit	The kit includes 1 CD and all reagents and consumables to generate 96 datapoints
P0021011	Gyrolab Mouse IFN-gamma Standard	Standard for spiking experiments, 80 000 pg/mL, 100 µL
P0021007	Gyrolab Mouse TNF-alpha Kit	The kit includes 1 CD and all reagents and consumables to generate 96 datapoints
P0021012	Gyrolab Mouse TNF-alpha Standard	Standard for spiking experiments, 112 000 pg/mL, 100 µL

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*The following products can be also purchased separately:*

Product Number	Product name	Description
P0021013	Gyrolab Biomarker Sample Dilution Buffer 2	Extra sample dilution buffer, 25 mL
P0020705	Gyrolab Bioaffy 4000 CD	1 Gyrolab Bioaffy CD microlaboratory, 4000 nL sample volume, 96 microstructures.
P0020999	Gyrolab Mouse IL-4 Kit Reagents	Includes capture and detect reagents, and standard stock solution required to generate 96 datapoints (1 CD)
P0021000	Gyrolab Mouse IL-6 Kit Reagents	Includes capture and detect reagents, and standard stock solution required to generate 96 datapoints (1 CD)
P0020998	Gyrolab Mouse IL-10 Kit Reagents	Includes capture and detect reagents, and standard stock solution required to generate 96 datapoints (1 CD)
P0021001	Gyrolab Mouse IFN-gamma Kit Reagents	Includes capture and detect reagents, and standard stock solution required to generate 96 datapoints (1 CD)
P0021002	Gyrolab Mouse TNF-alpha Kit Reagents	Includes capture and detect reagents, and standard stock solution required to generate 96 datapoints (1 CD)

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## Gyrolab Mouse Cytokine Kit content

Description	Gyrolab Mouse IL-4 Kit	Gyrolab Mouse IL-6 Kit	Gyrolab Mouse IL-10 Kit	Gyrolab Mouse IFN- gamma Kit	Gyrolab Mouse TNF- alpha Kit
Contents	P0021004	P0021005	P0021003	P0021006	P0021007
CD	1 x Gyrolab Bioaffy 4000	1 x Gyrolab Bioaffy 4000	1 x Gyrolab Bioaffy 4000	1 x Gyrolab Bioaffy 4000	1 x Gyrolab Bioaffy 4000
Capture Reagent	Biotinylated Anti-mouse IL-4 antibody, ready-to-use solution, 60 µL	Biotinylated Anti-mouse IL-6 antibody, ready-to-use solution, 60 µL	Biotinylated Anti-mouse IL-10 antibody, ready-to-use solution, 60 µL	Biotinylated Anti-mouse IFN-gamma antibody, ready-to-use solution, 60 µL	Biotinylated Anti-mouse TNF-alpha antibody, ready-to-use solution, 60 µL
Detection Reagent	Alexa Fluor™ 647 labeled Anti-mouse IL-4 antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-mouse IL-6 antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-mouse IL-10 antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-mouse IFN-gamma antibody, ready-to-use solution, 60 µL	Alexa Fluor™ 647 labeled Anti-mouse TNF-alpha antibody, ready-to-use solution, 60 µL
Standard	Stock solution of recombinant mouse IL-4 (48 ng/mL), 20 µL	Stock solution of recombinant mouse IL-6 (120 ng/mL), 20 µL	Stock solution of recombinant mouse IL-10 (1 µg/mL), 20 µL	Stock solution of recombinant mouse IFN-gamma (80 ng/mL), 20 µL	Stock solution of recombinant mouse TNF-alpha (112 ng/mL), 20 µL
Biomarker Wash Buffer 1	1500 µL	1500 µL	1500 µL	1500 µL	1500 µL
Biomarker Sample Dilution Buffer 2	25 mL	25 mL	25 mL	25 mL	25 mL
Gyrolab Wash Buffer pH 11	10 g	10 g	10 g	10 g	10 g

### Storage conditions:

Refrigerate at +4°C to +8°C.

### Shelf life:

See IFUs.

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