

Evaluating Infrared Carbon Dioxide Sensors for 21st Century Cell Culture: Introducing the Thermo Scientific IR180Si Infrared CO₂ sensor

Mary Kay Bates, M.S., Global Cell Culture Specialist, Earl Burris, Supervisor Product Specialist, Molly Love Parrucci, Global Product Manager, CO₂ Incubators, and Douglas Wernerspach, Global Business Director, CO₂ Incubators & Constant Temperature, Thermo Fisher Scientific

Key Words

CO₂ incubators, IR Sensor, Cell Culture

Abstract

In a cell culture incubator, carbon dioxide (CO₂) gas is provided to mimic mammalian physiological pH. Over the decades, different technologies have emerged to detect and control the amount of CO₂ gas entering the incubator, including older style constant flow, and popular thermal conductivity (TC) and infrared (IR) sensors. This article focuses on IR sensors to control CO₂. Different technologies which use infrared light do not produce the same results in the incubator and the differences are not commonly understood. We compare and contrast the available technologies, and highlight the advances incorporated in the new Thermo Scientific™ IR180Si CO₂ sensor. The IR180Si sensor with its silicon MEMS emitter is unique in that it will withstand many sterilization cycles. It eliminates the incandescent light bulb, which is the source of drift and limited lifespan for traditional IR CO₂ sensors. This unique design saves time and money for users due to much reduced need for calibration and replacement.

Introduction

A carbon dioxide (CO₂) incubator is the central equipment for any cell culture lab. When properly functioning and providing ideal growth conditions, the incubator ensures healthy, growing cells that allow myriad downstream applications including genetic analysis, protein expression and analysis, cell therapy, drug discovery, efficacy, toxicology and viability studies, and more. If, however, the incubator does not provide ideal conditions or is not properly calibrated, growth of the cells is negatively affected and all those functions – indeed, the normal operations and protocols of the laboratory — are inhibited, delayed or even halted completely.

A critical part of the CO₂ incubator is the monitoring and control of the carbon dioxide which functions to provide the optimal culture pH of 7.4 in concert with sodium bicarbonate or other buffer in the growth media. There are different technologies that measure and manage CO₂ in the incubator atmosphere, including constant flow,

thermal conductivity and infrared light. Thermo Scientific TC sensors remain widely popular worldwide, but this paper will focus on infrared (IR) sensors only.

IR monitoring of CO₂ is preferred in situations requiring GMP monitoring practices, increasingly used in therapeutic and production applications. IR sensors may be preferred in cases where the incubator door is opened frequently, such as for time course studies or where many users are sharing a single chamber.

However, just claiming that a given incubator offers an IR sensor is like stating you have a mobile phone. There are many different designs, and comparing them and understanding how design affects accuracy can be confusing. The choice is an important one because if the CO₂ system is not operating properly, cell growth, morphology and gene expression can be affected. In this article, we compare and contrast these different technologies, and introduce the new Thermo Scientific IR180Si sensor, providing data to demonstrate that this design provides increased accuracy and stability that lead to lifetime cost savings and overall better results.

Sensor positioning affects function

Before exploring design elements of IR sensors, let us consider why the position of the sensor within the incubator is critical. Some incubators place the sensor outside the incubation chamber in the electronics compartment. But an externally placed sensor will, by default, experience a delay in responding to conditions in the chamber. This is unavoidable because the air is extracted from the chamber by a pump and passed

through tubing to the sensor before returning to the chamber. As part of this process the air must be heated, otherwise the warm, 37 °C chamber air will condense into water on the cool, room temperature, external sensor, putting the electronics at risk and affecting the measurement accuracy.

Placing the sensor inside the incubator chamber gives more accurate results because an in-chamber location ensures the sensor experiences the same conditions as your cells. This also eliminates extra equipment including a pump, external heater, and tubing.

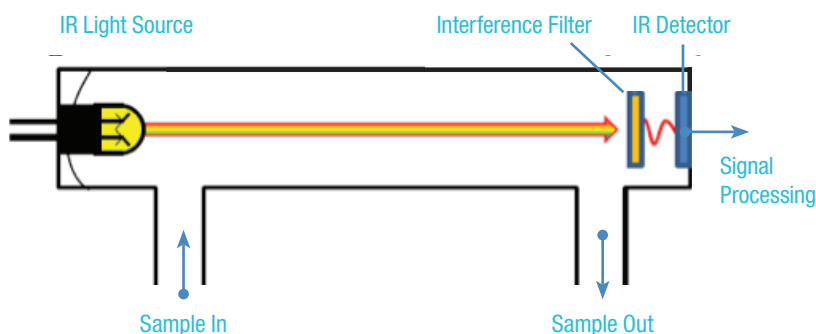
Infrared light is used to directly detect CO₂

A commonly used basic infrared sensor, shown in Figure 1, was introduced for the Thermo Scientific™ Forma™ CO₂ models in 1988. It consists of an incandescent bulb producing light including in the infrared band, a sample chamber, an interference filter and an IR detector. Gas enters the sample chamber and light is passed through it. CO₂ absorbs part of the IR light, at a characteristic wavelength of 4.3 μm. Figure 1 shows a non-dispersive IR (NDIR), where all the IR light passes through the gas sample and the light is filtered immediately before the detector. The interference filter allows only the selected wavelength through to the detection chamber, so the detector reacts only with light at the diagnostic CO₂ wavelength. So the amount of IR light measured at the detector opposite the IR source is a direct function of the amount of CO₂ gas present. If the measured result is too low, it triggers an influx of CO₂ to the incubation chamber.

Single beam, single wavelength infrared sensor

Figure 1 is an example of a single beam, single wavelength sensor. As shown, there is a single IR light source and a single detector. During calibration, a reference standard is measured, then removed. The electronics incorporate a mathematical correction based on the reference standard. For this to work well over time, the light source, electronics, filter and detector must remain stable and clean. Unfortunately, the accuracy of this sensor will drift over time. The drift occurs primarily because the light intensity is reduced as the incandescent tungsten filament ages. Also, dust and dirt can collect on the sensor surfaces, creating interference and appearing to the sensor as changes in the CO₂ concentration.

Figure 1: A basic infrared CO₂ sensor structure. The incandescent bulb generates light. Gas enters the chamber and interacts with the light. An interference filter blocks all light except at wavelength 4.3 μm, where CO₂ gas absorbs. That absorbance is then detected and processed to signal downstream effects.



Incorporating “Auto-Zero”

One approach to compensate for this inherent instability is to incorporate an auto-zero, or automatic background calibration method. At a specified interval, ambient air is pumped into the sensor chamber and measured. In general, an auto-zero is a big step forward. In practice, however, the auto-zero can be affected by a number of factors. Ambient CO₂ is increased with more humans in the room at a given time – all exhaling CO₂ – or reduced when the lab has been empty for hours. The ambient CO₂ can be reduced over time in labs with cement walls, because CO₂ spontaneously reacts with calcium hydroxide in the concrete, forming calcium carbonate and removing the CO₂ from the air¹. Barometric pressure changes can also affect the CO₂ reading according to Boyle’s law². An auto-zero, while establishing a base-line reading, is not really a calibration because it compares the chamber CO₂ of 5%, or 50,000 parts per million (ppm) to ambient air CO₂ of approximately 400 ppm, or 0.04%. Finally, the auto-zero does not correct for drift at 5% CO₂, where most cell culture incubators are set.

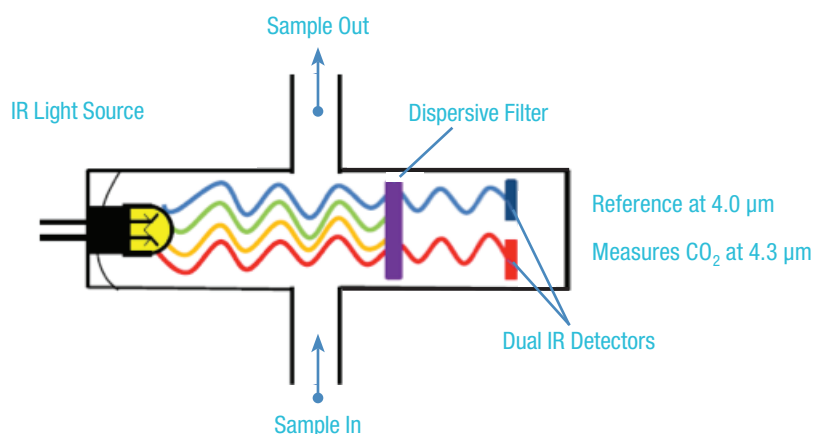
Dual beam, single wavelength infrared sensor

Another available technology incorporates a second IR light source, but this is rarely used in CO₂ incubators. The idea is that a second beam will compensate for drift as the primary light source ages, but since the second source will be subject to the same aging, it does not work well. Also, dust and dirt rarely accumulate evenly around the sensor and the different light sources, so the overall readings tend to be unreliable.

Single beam, dual wavelength infrared sensor

In an effort to provide increased accuracy and account for the inherent decreased light intensity as the IR bulb ages, some newer sensors use a single light source but measure a second wavelength produced by that same source as a reference. The first wavelength detects CO₂ concentration at 4.3 μm. The second wavelength measures at a reference band such as 4.0 μm, where CO₂ is not detected. So the reference wavelength accounts for change in light intensity over time, because it provides a zero that is subject to the same conditions as the detection wavelength. This is a huge improvement that provides increased stability. It also eliminates the need for any complicated algorithms to compensate for variation, and eliminates the need for an auto-zero and pump to sample ambient air.

Figure 2: A single beam, dual detector IR sensor. The measuring and reference wavelengths are measured at separate detectors. The different detectors are subject to different optical paths and conditions.



Single beam, dual detector infrared sensor

Once a second wavelength is incorporated, there has to be a way to detect it. One approach is to add a second detector. This design is depicted in Figure 2. While this strategy has the benefits of the second reference wavelength it is subject to some of the same faults as the dual beam, single wavelength sensor with two light sources. This light source will still burn out over time and the light intensity will decrease. In addition, because the reference detector is separate from the CO₂ measuring detector, it will not correct for dust and dirt, which impact the reference detector differently than the measuring detector, and two detectors means two optical paths with different conditions. Thus, using two detectors does not provide a true reference.

Introducing the Thermo Scientific IR180Si Infrared CO₂ Sensor

Thermo Scientific™ Forma™ Steri-Cycle™ and Heracell™ VIOS CO₂ incubators feature the IR180Si CO₂ sensor, which incorporates new technology to address drawbacks of earlier IR sensors. This sensor is uniquely able to withstand the 180°C Thermo Scientific Steri-Run™ automated sterilization cycle. It also replaces the traditional incandescent light bulb with a silicon MEMS emitter, and uses a tunable Fabry-Perot Interferometer (FPI) filter with a single detector.

Single beam, dual wavelength, single detector infrared sensor

The IR180Si sensor uses a single beam with dual wavelengths, but instead of using two detectors and a standard dispersive filter, the IR180Si sensors use a micromachined FPI filter in front of a single detector. The FPI filter has a tiny electronic chip which is electrically tuned to constantly shift between the CO₂ detection wavelength and the reference wavelength, providing real-time calibration of the measurement. This approach solves three problems; it compensates for changes in the IR light intensity as well as for dirt and dust accumulating in the light path, without adding another electronic piece in the form of a second detector.

Also, since the FPI filter is electrically tuned between the reference and measuring wavelengths, it has no moving parts that could break down, such as that used in a rotating filter wheel design.

Next generation technology eliminates the infrared light bulb

Nearly all of the IR CO₂ sensors available today use a tiny incandescent light bulb as the source of the infrared light. The light intensity can vary greatly among sensors. The reason for this inherent instability is that the thin tungsten filament continually gives off tungsten, which accumulates on the inside of the glass bulb, dimming the light output. Also, that shedding tungsten means the filament continually thins, resulting in decreasing light output. This explains how the incandescent IR light, like any light bulb, will eventually burn out.

Introducing a new infrared light source: silicon

The new Thermo Scientific IR180Si sensor incorporates a silicon micro-electrical mechanical systems (MEMS) emitter as the infrared light source, as shown in Figure 3.

Figure 3: Design of the new Thermo Scientific IR180Si CO₂ sensor, incorporating a Silicon MEMS light source, a tunable FPI filter and a single detector. The FPI filter constantly switches between the two wavelengths, providing real time calibration for a true measurement by a single detector. The silicon chip light source is projected to last at least 50% longer than an incandescent bulb.

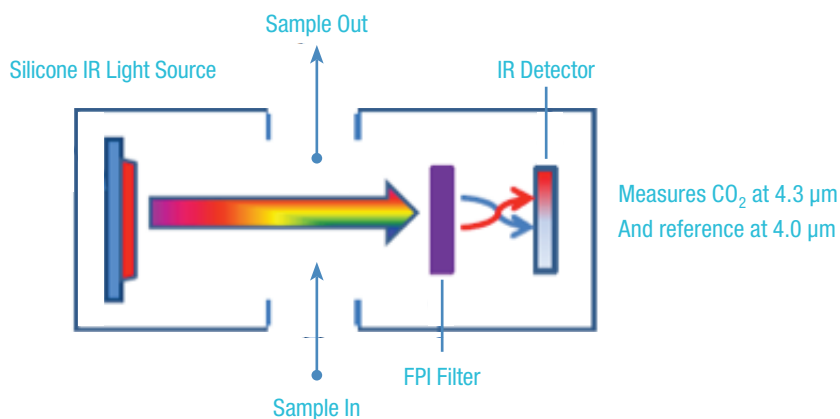
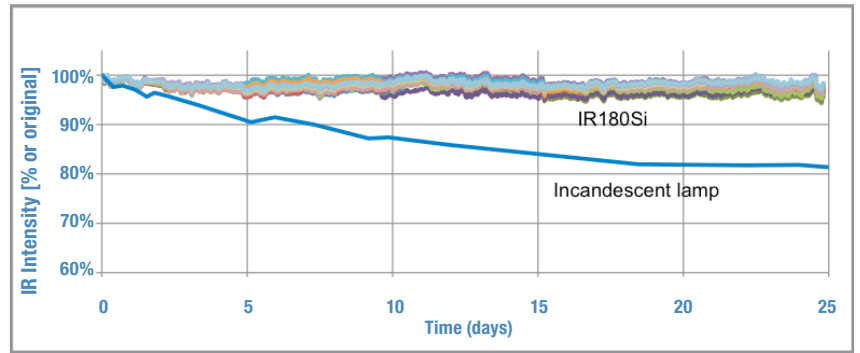


Figure 4: Stability of IR180Si infrared light intensity over time, compared to an incandescent light. N= 23 for IR180Si sensors tested.



MEMS technology provides precision that delivers outstanding performance and uniformity, but also saves money due to batch fabrication techniques and lower power consumption³. The IR180Si sensor is projected to have a much longer lifetime than traditional incandescent IR sensors. Figure 4 compares the stability over time of the silicon IR180Si light source to that from a typical incandescent IR light bulb with a tungsten filament. The IR180Si light is highly stable over time, especially compared to the incandescent light, which shows steadily decreasing light intensity. The robust IR180Si is projected to have at least a 50% increased lifespan compared to sensors with an incandescent light source, minimizing replacement costs (manufacturer data).

The IR180Si sensor shows outstanding stability

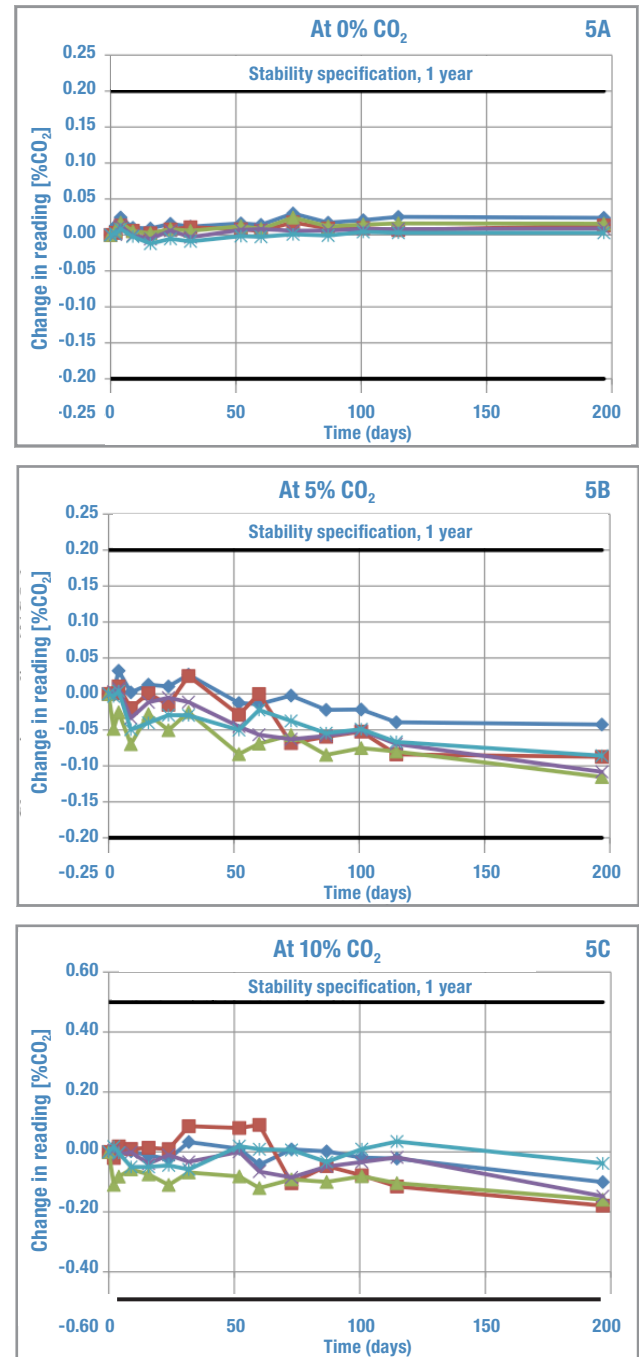
Stability of the IR180Si sensor remains well under specifications at all CO₂ settings

Sensors will often look great in data at a single setpoint but in a working CO₂ incubator, it is not uncommon to require different CO₂ settings over the life of the lab. Some cells are cultured in DMEM at 5%, some at 10%, and some are cultured in closed systems and gassed separately such that CO₂ is not required in the incubator. But with increasing CO₂ concentration, the accuracy of the sensor decreases, since the reference is essentially zero. So it is instructive to investigate the behavior of a sensor over time, under different CO₂ concentrations. As shown in Figure 5, the IR180Si sensor remains remarkably stable over the course of 197 days and well within prescribed specifications (manufacturer data). The outstanding performance far exceeds standard specifications of 0.2% drift at 0% and 5% CO₂, and 0.5% drift at 10% CO₂. Six different sensors were tested.

The IR180Si sensor will withstand automated high temperature sterilization cycles

In addition to the amazing stability and long life of the IR 180Si CO₂ sensor, there is another enormous benefit to its design. Because of the robust MEMS electronics and the stable silicon light source, the sensor can remain in the incubator chamber throughout multiple high temperature sterilization cycles. The Thermo Scientific Forma Steri-Cycle i series and Heracell VIOS CO₂ incubators feature the Steri-Run™ cycle, an automated 180 °C sterilization. In both, the CO₂ sensor is positioned in the chamber, to react to the same conditions that your cells are experiencing and that sensor never

Figure 5: The IR180Si CO₂ sensor shows outstanding stability over time. (5A) 0% CO₂, (5B) 5% CO₂, and (5C) 10% CO₂ all fall well within standard specifications of 0.2% drift at 0% and 5% CO₂, and 0.5% drift at 10% CO₂, over the course of 197 days. N=6 sensors tested.



has to be removed. As shown in Figure 6, even after 137 automated cycles, the sensor is still well within the specifications for drift at 0%, 5% and 10% CO₂ (manufacturer data). Six different sensors were tested. Eliminating handling of the sensor reduces chances of breakage or introduction of contamination that could put cells at risk.

Conclusions

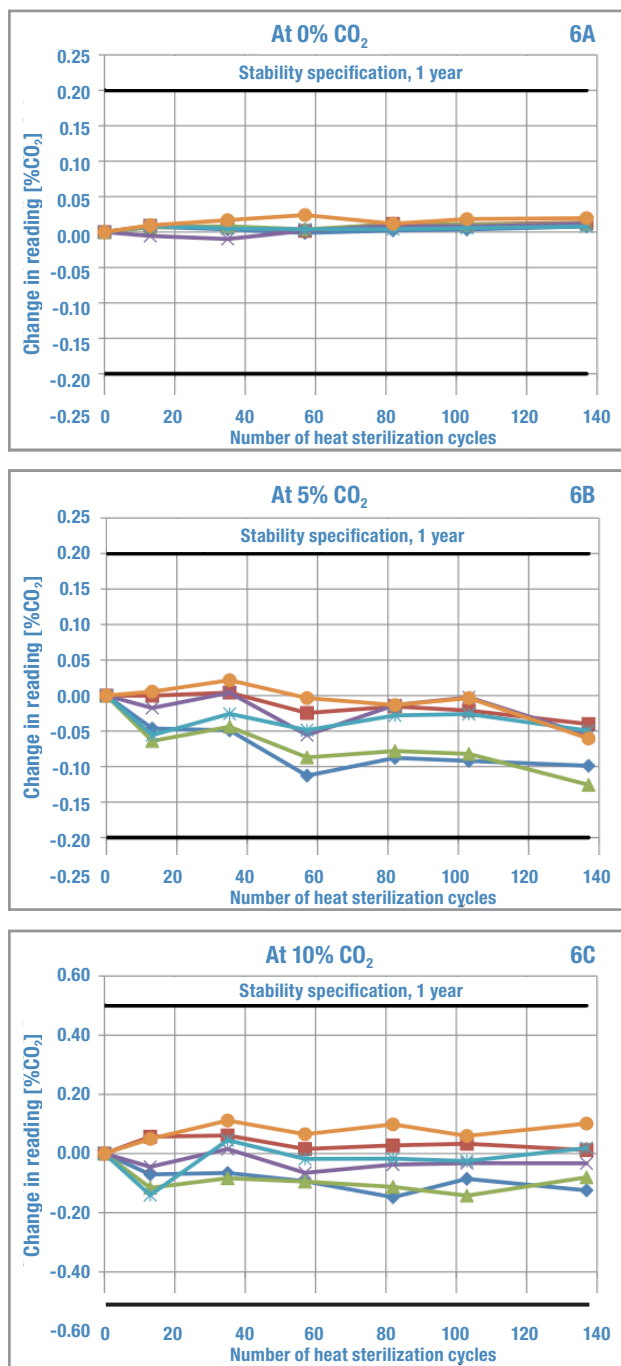
IR CO₂ sensors are available in various types and most have inherent disadvantages that are not apparent to the casual observer. This article explains the differences and explains why positioning a sensor in the incubation chamber provides the best measurement. The new IR180Si CO₂ sensor available for Thermo Scientific Forma Steri-Cycle i series and Heracell VIOS CO₂ incubators is shown to be amazingly stable. It remains well within specifications at different CO₂ concentrations and over time. Because of its robust silicon MEMS light source and design, it can remain *in situ* even during multiple high temperature sterilization cycles, eliminating any handling or risk of contamination. The silicon light source provides stability far improved over the traditional incandescent infrared light bulb, which means a vastly improved life span. The non-dispersive FPI filter and single detector for the dual wavelengths eliminate variability, providing a true reference. The IR180Si sensor represents an unmatched step forward in CO₂ sensor design.

The IR180Si accuracy and extended lifespan marry perfectly with onboard and remote monitoring programs for GMP applications. Thermo Scientific CO₂ incubators offering the IR180Si sensor are ideally suited for use in cutting edge cell culturing applications including stem cell culture and cell therapy for clinical research applications, embryo cultures from in vitro fertilization (IVF) procedures, primary cell culturing and more.

References

1. **Concrete Experts International.** Carbonation of concrete. Concrete Experts International. [Online] 8 21, 2006. <http://www.concrete-experts.com/pages/carb.htm>.
2. **Tro, NJ.** *Principles of chemistry, a molecular approach.* Boston : Pearson, 2013.
3. **MEMS and Nanotechnology Exchange (MNX).** MEMSnet. [Online] Corporation for National Research Initiatives. www.memsnet.org.

Figure 6: The IR180Si CO₂ sensor shows outstanding stability at (6A) 0% CO₂, (6B) 5% CO₂, and (6C) 10% CO₂, even after withstanding 137 Steri-Run sterilization cycles. Specifications are for 0.2% drift at 0% and 5% CO₂, and 0.5% drift at 10% CO₂. N=6 sensors tested.



thermoscientific.com/co2

© 2014 Thermo Fisher Scientific Inc. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

Australia +61 39757 4300
Austria +43 1 801 40 0
Belgium +32 53 73 42 41
China +800 810 5118 or
 +400 650 5118
France +33 2 2803 2180
Germany national toll free 0800 1 536 376
Germany international +49 6184 90 6000

India toll free 1800 22 8374
India +91 22 6716 2200
Italy +32 02 95059 552
Japan +81 3 5826 1616
Netherlands +31 76 579 55 55
New Zealand +64 9 980 6700
Nordic/Baltic/CIS countries
 +358 9 329 10200

Russia +7 812 703 42 15
Spain/Portugal +34 93 223 09 18
Switzerland +41 44 454 12 22
UK/Ireland +44 870 609 9203
USA/Canada +1 866 984 3766

Other Asian countries +852 2885 4613
Countries not listed +49 6184 90 6000

Thermo
 SCIENTIFIC

A Thermo Fisher Scientific Brand