

PCR-Campylobacter Detection Kit

(Catalog # K1453-96; 96 Rxns; Storage at -20°C)

I. Introduction:

Thermophilic Campylobacter species (mainly *C. jejuni*, *C. coli*, *C. upsaliensis* and *C. lari*) are causal agents of enteritis and may be found as commensal organisms in the gastrointestinal tract of a wide range of domestic and farm animals.

PCR-Campylobacter Detection kit is an ideal tool for a specific, rapid, and reliable detection of thermophilic Campylobacter (*C. jejuni*, *C. coli*, *C. upsaliensis* and *C. lari*) from food and environmental samples by real time PCR. The Kit includes all the reagents required in a comfortable ready-to-use PCR MasterMix. The optimized MasterMix contains a Buffer, dNTPs, Hot-start DNA Polymerase, DNA-free water, MgCl₂ and an Internal Amplification Control (IAC) whose detection indicates the absence of PCR inhibitors. Primers and Probes for the amplification of IAC as well as for the amplification of the target gene are included in the MasterMix. The probe for the detection of target gene is labeled with the FAM, whereas the probe for the detection of IAC is labeled with the JOE fluorochrome.

Additionally, the kit includes both Positive Control and Negative Controls. The Positive control is supplied to demonstrate that the PCR amplification is working efficiently with the supplied components. To confirm absence of contamination, a Negative control reaction should be included every time the kit is used. The kit also includes DNAREady Lysis Buffer to extract the DNA from the sample prior to PCR Detection.

II. Applications:

- An ideal tool for specific, rapid, and reliable detection of thermophilic Campylobacter (*C. jejuni*, *C. coli*, *C. upsaliensis* and *C. lari*) from food and environmental samples by real time PCR.

III. Sample Type:

- Food, water and environmental samples

IV. Kit Contents:

Components	K1453-96	Part Number
PCR Master Mix	1 Vial	K1453-96-1
PCR Positive Control	1 Vial	K1453-96-3
PCR Negative Control	1 Vial	K1453-96-4

V. User Supplied Reagents and Equipment:

- PCR tube or plate well
- Centrifuge
- Thermal cycler

VI. Storage Conditions and Reagent Preparation:

All the reagents are shipped in dry ice and stored at -20°C upon receipt. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months.

VII. Assay Protocol:

- Centrifuge 1 ml of the enrichment for 5 minutes at 8000g. Discard the supernatant and extract the DNA from the pellet using the method of choice.
- Place 19 µl of the Reaction Mix into each PCR tube or plate well. Perform this operation in a clean environment protected from light.
- Load 1 µl of the extracted DNA samples into each PCR tube or plate well. Load also 1 µl of the positive controls or non-template controls (NTC) into the appropriate tubes or plate wells.
- Place the PCR tubes or the plate into the real time thermal cycler. Set the fluorescence reading at the channels corresponding to the fluorochromes FAM and HEX. Use the following program to perform the amplification:

Step	Event	Temperature	Time
1	DNA polymerase activation and DNA denaturation	95°C	10 minutes
2 (40 Cycles)	Denaturation	95°C	15 seconds
	Annealing/Extension	60°C	1 minute*

*Fluorescence measurements: FAM: Campylobacter; HEX: Internal Amplification Control.

5. Read the results.

CONTROL REACTIONS:

It is highly recommended to perform at least one non-template control (using 1 µl of sterile DNA-free water instead of DNA) and one positive control (using the included PCR Positive Control or genomic DNA from Campylobacter) in each PCR run.

VIII. Related Products:

- PCR Master Mixes and Kits (Cat# M1127- M1145)
- DNA Extraction (Cat# K1411-K1417); K309; K316
- Q-PCR (Cat# M1105-M1126)

FOR RESEARCH USE ONLY! Not to be used on humans.