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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. The kit is based on the detection by real time PCR of three target genes with a specific sequence for Campylobacter species. After the enrichment step at 42°C, a positive amplification indicates the presence of thermophilic Campylobacter (C. jejuni, C. coli, C. upsaliensis, C. lari. and some others) in the sample. The kit can also be used to confirm colonies growing on agar plates at 42°C.

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, $MgCl_2$ 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 HEX fluorochrome. The Reaction Mix does not contain ROX. Omit the use of this fluorophore during the setup of the real time PCR run for instruments with a passive reference dye system or add the ROX dye to the Reaction Mix at the concentration specified for the instrument.

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 DNA 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	2 Vials	K1453-96-1
PCR Positive Control	1 Vial	K1453-96-2
PCR Negative Control	1 Vial	K1453-96-3
DNA lysis buffer	2 Vials	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:

1. 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.
2. Place 19 µl of the Reaction Mix into each PCR tube or plate well. Perform this operation in a clean environment protected from light.
3. 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.
4. 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*

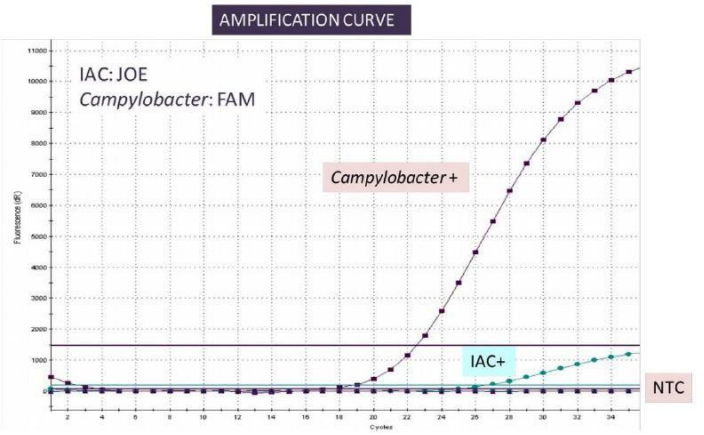
**Fluorescence measurements: FAM: Campylobacter; HEX: Internal Amplification Control.*

5. Read the results.
6. Several assays have been performed using this kit with a large range of bacteria without obtaining cross-reactions. However, it could produce cross-reactions with some Arcobacter species, especially if enrichments are not carried out at 42ºC.

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.

RESULTS INTERPRETATION



A sample will be considered positive whenever the fluorescence corresponding to Campylobacter (FAM) is higher than the threshold value. A sample will be considered negative only when the fluorescence of Campylobacter remains below the threshold value but the fluorescence of IAC (HEX) increases over the threshold value

Note: Fluorescence levels of may be distinct for every channel. This may depend on the optical configuration of the thermocycler used, as well. Usually, FAM fluorescence values are higher than those of HEX.

PROBLEM	CAUSE	SOLUTION
Neither Campylobacter nor IAC specific signals are detected.	PCR inhibition	Dilute the sample 10 and 100-fold and repeat the analysis or use a DNA purification kit to remove the inhibitors.
	Bad storage of the Reaction Mix	Store the Reaction Mix at the recommended temperature and avoid contact with light. Check expiration date.
Campylobacter specific signal is obtained but no IAC signal is detected	Preferential amplification of the *Campylobacter* DNA due to a high abundance of this DNA in the sample	The reaction is satisfactory and positive for *Campylobacter.*
Campylobacter specific amplification is detected in non-template controls	Contamination of materials or reagents	Repeat the analysis with fresh reagents and cleaned pipettes. Wash surfaces with freshly diluted bleach (10%) or a similar reagent.
Campylobacter specific amplification is obtained in non-template controls but no IAC signal is detected.	Contamination of materials or reagents and preferential amplification of the Campylobacter DNA due to a high abundance of this DNA or due to a problem with IAC amplification	If other tubes present a positive IAC amplification, the problem is not related to IAC amplification.
		Repeat the analysis with fresh reagents, cleaned pipettes and surfaces washed with freshly diluted bleach (10%) or a similar reagent.
No specific amplification of *Campylobacter* is obtained in positive control tubes	If no IAC signal is observed: bad storage of the Reaction Mix	Store the Reaction Mix at the recommended temperature and avoid contact with light. Check expiration date
	If IAC signal is detected: pipetting error or positive control degradation.	Repeat the analysis and ensure that an appropriate positive control is added into the corresponding tubes.

VIII. Related Products:

BioVision Product Name	Cat. No.	Sizes
PCR-Salmonella Detection Kit	K1447	96 Rxns
PCR-Salmonella-Listeria Detection Kit	K1448	96 Rxns
PCR-Listeria monocytogenes Detection Kit	K1449	96 Rxns
PCR-Legionella spp Detection Kit	K1450	96 Rxns
PCR-Legionella spp Plus Detection Kit	K1451	96 Rxns
PCR-STEC Detection Kit	K1452	96 Rxns

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