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DATA SHEET

Version: 03
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1. Identification

Product name

PRImeDETECT™ Campylobacter Detection Kit

(Thermophilic Campylobacter species)

Cat. No **FP0041** (96 reactions)

2. Description

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.
PRImeDETECT™ Campylobacter Detection Kit provides a quick, reliable, sensitive, and highly specific method to detect Campylobacter species in a single reaction tube by performing a real time PCR. **PRImeDETECT™ Campylobacteris** 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.
All reagents required for qPCR are provided ready to use as PCR Master Mix (1 vial). The PCR Master Mix contains the appropriate amounts of buffer, dNTPs, Hot-start DNA polymerase, DNA-free water and MgCl2 to perform the number of reactions indicated in the kit. The PCR Master Mix also includes an internal amplification control (IAC) whose detection indicates the absence of PCR inhibitors. The probe for the detection of Campylobacter is labelled with FAM, whereas the probe for the detection of IAC is labelled 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. In addition, the kit includes positive control DNA and negative control. 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

3. Composition

✓ PCR Master Mix (1 vial)

✓ PCR Positive Control (1 vial)

✓ PCR Negative Control (1 vial)

4. Specific characteristics

- ✓ Easy to use.
- ✓ Highly conserved target genes, reduce false negative results.
- ✓ High inclusivity and exclusivity for thermophilic Campylobacter species (*C. jejuni*, *C. coli, C. upsaliensis* and *C. lari*).
- ✓ Amplification limit of one copy per reaction.
- ✓ Without the need to use tedious conventional isolation and identification techniques.
- ✓ Reliable results in c.a. 48 hours, including the enrichment step.
- ✓ Easy interpretation of the results from the amplification curves.
- ✓ Internally validated with a wide variety of food samples.

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5. Storage specifications

The **PRImeDETECT™ Campylobacter Detection Kits** are shipped at ambient temperature. On arrival the kit should be stored at -20˚C. Avoid prolonged exposure to light. If stored correctly the kit will retain full activity for 12 months. The kit can be stored at 4˚C for 1 month. PLEASE, CHECK REAGENTS INTEGRITY BEFORE USE. THE USE OF DETERIORATED REAGENTS MAY LEAD TO UNRELIABLE AND/OR EQUIVOCAL RESULTS.

6. Further information

Product Use Limitations This product is developed, designed, and sold exclusively only for research purposes use. The product was not tested for use in diagnostics or for drug development, nor is it suitable for administration to humans or animals.

Safety Information When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at www.canvaxbio.com where you can find, view, and print the MSDS for each CANVAX kit.

PROTOCOL

1. Centrifuge 1 ml of the enrichment for 5 minutes at 8000 × g. 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 nontemplate 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. 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.

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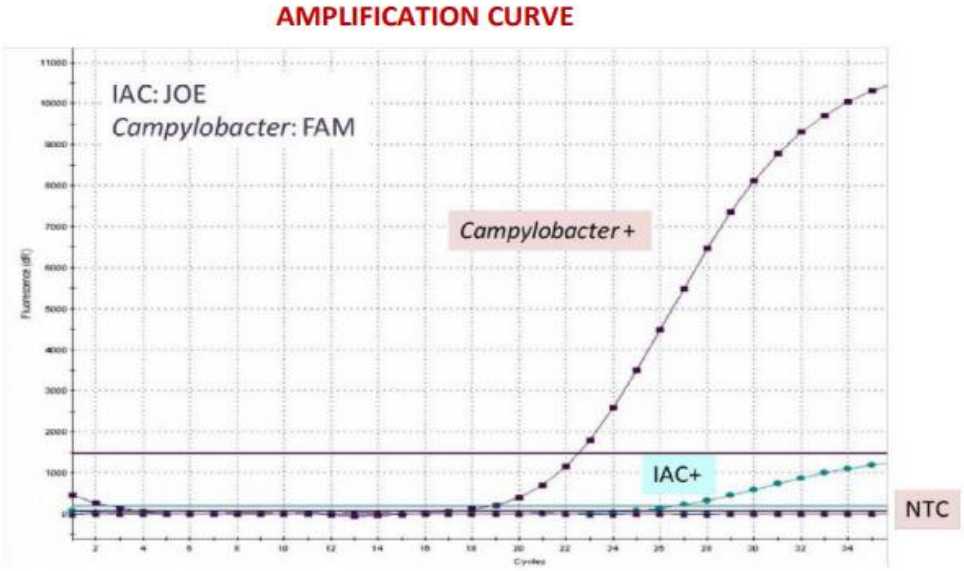
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7. Procedural Precautions

1. Laboratory DNA extraction and amplification areas, as well as materials, equipment and reagents, should NOT be used for other activities or transferred from one area to another. Discard gloves used in one area and use new gloves in the next area.
2. Good Laboratory Practice must be observed to obtain reliable results with this technique. The high sensitivity of this test requires extreme care to maintain the purity of all reagents. Discard all suspicious reagents.
3. Nucleic acids are very sensitive to degradation by nucleases, which are present in human skin and in surfaces that have been in contact with human skin. Wash surfaces with appropriate reagents, use powder-free examination gloves and a lab coat throughout the whole test. Wash hands thoroughly after performing the test.
4. This test has been validated by using the reagents provided with PRImeDETECT™ Campylobacter. The use of other amplification methods or any change in the protocol may render false results. DO NOT INTERCHANGE COMPONENTS from different lots.
5. Do not use **PRImeDETECT™ Campylobacter** after expiry or best before date. Store this product at the indicated temperature and conditions.
6. The use of this product is limited to qualified personnel experienced in DNA extraction and amplification techniques.
7. This test has been designed to investigate the presence of thermophilic Campylobacter in water and food samples, or for other purposes related to R&D. Do NOT use **PRImeDETECT™ Campylobacter** as a diagnostic tool in clinical samples.
8. Several assays have been performed using **PRImeDETECT™ Campylobacter** 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.

8. 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.*

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9. Troubleshooting Guide

PROBLEM	CAUSE	SOLUTION
Neither Campylobacter nor IAC specific signals are detected.	PCR inhibition	Dilute the sample 10 and 100-fold and repeat the analysisor use a DNA purification kit to remove the inhibitors
	Bad storage of the ReactionMix	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 issatisfactory 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
		Repeat the analysis with a new tube of PRImeDETECT™ Campylobacter
		If contamination persists contact our Technical Department
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 ofthis DNA or due to a problem with IAC amplification	If other tubes present a positive IAC amplification,the problem is not related to IACamplification
		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 expirationdate
	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