コスモ・バイオ株式会社

Dinophysis spp **PCR detection Kit**

Product Number # MBK0008

50 REACTIONS

Store at -20°C

Dinophysis spp. **detection kit for use with PCR Thermal Cyclers**

INTENDED USE

Identification of *Dinophysis spp.* DNA by polymerase chain reaction (PCR).
FOR LABORATORY USE ONLY

INTRODUCTION

Diarrhoeic shellfish poisoning (DSP) is a syndrome predominantly caused by the consumption of contaminated shellfish. DSP toxins are produced by dinoflagellates such as *Dinophysis spp.* Coastal water monitoring programmes to detect the presence of toxic algae could provide an essential tool to access bloom formation and consequently potential shellfish contamination. PCR-based methods are attractive tools for coastal water monitoring programmes because they can rapidly detect as low as one cell of specific organism in mixed ecological scenarios. These methods have been successfully applied to seawater samples for the identification of several Dinoflagellates, reducing the time for the standard monitoring procedures (microscope observation).

PRODUCT DESCRIPTION

The "*Dinophysis spp.* PCR detection Kit" allows the detection of *Dinophysis* DNA using polymerase chain reaction (PCR). PCR primers specifically detect all the Dinophysis species. The kit contains reagents and enzymes for the specific amplification of a 366 bp region of the *Dinophysis* genome. This PCR assay is sensitive enough to detect up to one cell per reaction. To check for possible PCR inhibition, an internal control, giving an amplicon of 214 bp, is also supplied.

KIT CONTENTS

Instruction manual

Dinophysis **spp. Mix:**	2000 µl (2 x 1000 µl) containing primers, dNTPs, 10X reaction buffer, $MgCl_2$ and an internal control (plasmid DNA including specific primer complementary sequences)
Positive control:	1000 µl (50 $x10^9$ copies of plasmid DNA containing *D.sacculus* specific sequence)
Hot-Rescue DNA Polymerase:	10 µl

STORAGE TEMPERATURE
Store at -20°C. Repeated thawing and freezing may reduce the sensitivity and should be avoided. It is suggested to frozen the reagents in aliquots for intermittently use.

GENERAL PRECAUTIONS FOR PCR
The user should always pay attention to:

- use pipette tips with aerosol-preventive filters, deionized DNA-free water and gloves;
- store positive material (specimens, controls and amplicons) separately from all other reagents and, if possible, add it to the reaction mix in a separated space;
- do not use the same precision pipettes for reaction mix and DNA;
- thaw all components samples at room temperature before starting an assay;
- when thawed, mix the components and centrifuge briefly.

PROCEDURE

DNA ISOLATION
Various protocols (e.g. phenol-chloroform, CTAB) or kits (e.g. Diatheva-Dinoflagellate DNA isolation Kit prod. n. MBK0007) can be use to extract phytoplankton DNA. Carry out the DNA isolation according to the procedure in order to avoid contamination from PCR inhibitors (e.g. humic acid).

PCR SET UP

Dilution of the positive control
Make fresh dilutions before every PCR to avoid plasmid degradation
1) Mix by vortexing, take 5 µl of the control stock solution and add 995 µl deionized, DNA-free water
2) Mix by vortexing, take 10 µl of the first dilution and add 990 µl deionized, DNA-free water
3) Mix by vortexing, take 10 µl of the second dilution and add 990 µl deionized, DNA-free water, to obtain the final 1:2,000,000 working dilution.

PCR master mix:
Total volume per reaction is 50 µl.
Include a positive control (option) and at least one negative control (water) in each PCR run.
Before each use thaw all reagents completely, mix and centrifuge.
Pipet mastermix on ice into a 1.5 ml reaction tube and mix (see table 1)

Table 1

	1 reaction	25 reaction
Dinophysis spp mix	39.8 µl	995 µl
Taq Diatheva (5U/µl)	0.2 µl	5 µl
Total volume	40 µl	1000 µl

Aliquot 40 µl of master mix into each PCR reaction tube before adding 10 µl sample DNA*, negative (water) and positive control.
*It is preferable to employ 10 ng DNA/reaction but up to 1 µg of good quality purified DNA can be amplified without inhibitory effects

After pipetting the negative control and the samples, the tubes must be sealed in order to avoid cross contamination during the addition of positive control.
Centrifuge briefly
For PCR thermal cycler without heated lid each reaction mixture will have to be covered with a drop of mineral oil.

THERMAL PROFILE
Program the PCR thermal cycler with the following parameters:

1 cycle	95°C for 10 min
35 cycles	95°C for 30 sec 58°C for 30 sec 72°C for 30 sec
1 cycle hold	72°C for 7 min 4°C

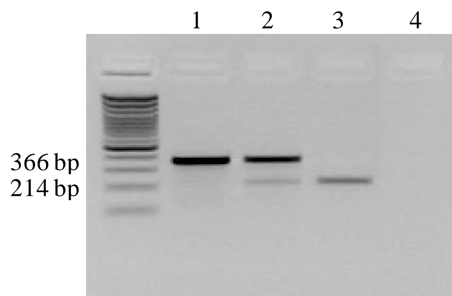
AGAROSE GEL ELECTROPHORESIS
Mix 20 µl of the PCR reaction with loading buffer. Separate the DNA in the presence of a DNA standard specific for the low range (100-1000 bp), on a 2% agarose gel, containing ethidium bromide. Stop electrophoresis after 3-cm run distance.

DATA ANALYSIS
If internal control DNA was used, a distinct 366 bp band should appear in every lane indicating a successfully performed PCR. This band may fade out with increased amount of amplicons formed.

BAND PATTERN INTERPRETATION

Band Pattern	Interpretation
Band at 214 bp	Negative sample
Band at 366 bp with possible band at 214 bp	Positive sample
No band	No Diagnosis (See Troubleshooting section)

Example of positive and negative samples are showed in the following gel electrophoretic separation of PCR products:



1) one band 366 bp: positive sample, strong contamination
2) one band 366 bp + one faint band 214 bp: positive sample
3) one band 214 bp: negative sample
4) no band: inhibited sample

TROUBLESHOOTING

No amplification of controls DNA may be due to the following reasons:

1. Incorrect programming of the thermal cycler: repeat the PCR with the correct settings.
2. Pipetting mistake: check pipetting and repeat the PCR.
3. Degraded reagent: store PCR reagents at -20°C and keep on ice once thawed. Avoid multiple freeze-thaw cycles.
4. PCR reaction inhibition: repurify the DNA sample to remove inhibitors.

The presence of the band of 214 bp in the negative control is due to contamination of PCR reaction: vigorous cleaning is recommended before repeat the amplification.