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Alexandrium spp. PCR detection Kit

MBK0003
50 Reactions

Intended use

Identification of *Alexandrium* spp. DNA by polymerase chain reaction (PCR).

Introduction

Paralytic shellfish poisoning (PSP) is a syndrome predominantly caused by the consumption of contaminated shellfish. PSP toxins are produced by dinoflagellates such as *Alexandrium* spp. Coastal water monitoring programmed to detect the presence of toxic algae could provide an essential tool to access bloom formation and consequently potential shellfish contamination. Usually, monitoring involves accurate morphological identification and the enumeration of target phytoplankton species in the seawater samples by using standard microscopy procedures. These methods are time-consuming and require a remarkable taxonomic experience. PCR-based methods are actractive as diagnostic tools 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.

Product description

The "*Alexandrium spp.* PCR detection Kit" allows the detection of *Alexandrium* DNA using polymerase chain reaction (PCR). PCR primers specifically detect all the *Alexandrium* species. The kit contains reagents and enzymes for the specific amplification of a 135 bp region of the *Alexandrium* 5.8S rDNA. This PCR assay is able to detect down to one cell per reaction. To check for possible PCR inhibition, an internal control, giving an amplicon of 217 bp, is also supplied.

Kit contents

2 x 1ml *Alexandrium* spp. Mix - containing primers, dNTPs, 10X reaction buffer, $MgCl_2$ and an internal control plasmid DNA including specific primers.

1 x 0,5ml *Alexandrium* Positive Control - DNA containing A. *catenella* specific sequence

1 x 10 µl Hot-Rescue DNA Polymerase

1 x 1ml PCR-grade Water

Storage

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

Precautions

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 commercial kits (e.g. Diatheva, *Dinoflagellate DNA isolation Kit* Code: 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

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

Table 1

Component	For 1 reaction	25 reaction
Alexandrium spp. mix	39.8µl	995µl
Hot Rescue DNA Polymerase (5U/ µl)	0.2µl	5µl
Total volume	**40µl**	**1000µl**

Aliquot 40µl of mix into each PCR reaction tube before adding 10µl of DNA sample*, negative control (10µl water) and positive control (2µl provided ***Alexandrium*** **Positive Control** + 8µl water).

*It is preferable to employ 10ng DNA/reaction but up to 1µg of good quality purified DNA can be amplified without inhibitory effects.

After preparing the controls and the samples, the tubes must be sealed in order to avoid cross contamination during the addition of positive control.
Centrifuge briefly.

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

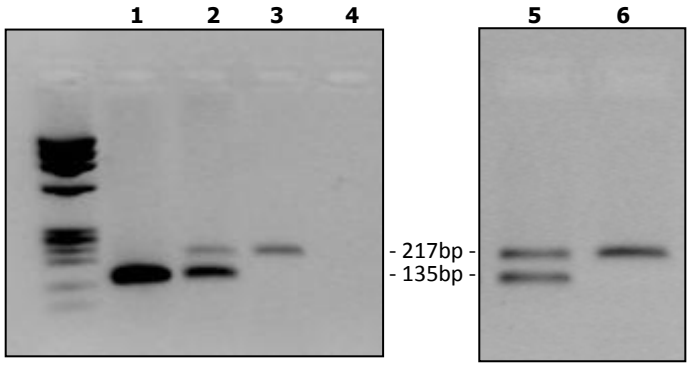
Mix 20µl of the PCR reaction with loading buffer. Separate the DNA in the presence of a DNA standard specific for the low range (100-1000 bp) on a 3% agarose gel, containing ethidium bromide or any other stain gel agent.

Data analysis

The internal control DNA allows to obtain a distinct 217 bp band in every lane indicating a successfully performed PCR. This band may fade out with increased amount of specific amplicons formed.

Band pattern	Interpretation
Band at 217 bp	Negative sample
Band at 135 bp with possible band at 217 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 135 bp: positive sample, strong contamination
2) one band 135 bp + one faint band 217 bp: positive sample
3) one band 217 bp: negative sample
4) no band: probable presence of PCR inhibitors in the DNA sample

5) *Alexandrium* Positive Control
6) Negative Control

Troubleshooting

No amplification in the positive control tube may be due to the following reasons:

- Incorrect programming of the thermal cycler: repeat the PCR with the correct settings.

- Pipetting mistake: check pipetting and repeat the PCR.

- Degraded reagent: store PCR reagents at -20°C and keep on ice once thawed. Avoid multiple freeze-thaw cycles.

If PCR inhibition is observed: re-purify the DNA sample to remove inhibitors.