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Dinoflagellate DNA isolation Kit

Magnetic purification of Genomic DNA from **fixed** dinoflagellate samples

MBK0007

50 Reactions

Intended use

The "Dinoflagellate DNA isolation Kit" is optimized for the isolation of total genomic dinoflagellate DNA from fixed phytoplankton samples. DNA purified with this method is suitable for PCR reactions.

Introduction

Traditionally, phytoplankton samples collected for marine monitoring programmes or aquatic microrganism studies are preserved with fixative substances for long-term storage before analytical treatments such as microscope-based cell identification or simply to permit the transport from one laboratory to another. In recent years, to simplify microalgal identification and enumeration, the use of DNA or RNA probes coupled with the PCR assay has progressed and now represents a routine application for screening cultured and field samples. The efficiency of the genomic DNA extraction step is very important for the subsequent PCR assay, especially when it has to be used in quantitative investigation on cultured or environmental samples. In fact, the efficiency of amplification is guaranteed by complete cellular lysis and careful purification of target DNA (1, 2).
This kit permits the rapid isolation of total genomic DNA working directly with the fixed phytoplanktonic sample. The purified DNA is suitable for PCR amplification without problems of inhibitions.

Product description

The "Dinoflagellate DNA isolation Kit" allows the extraction of Genomic DNA from fixed phytoplankton samples using Magnetic beads (paramagnetic nanoparticles coated with silica). (4%) is enzimatically digested using Proteinase K and optionally submitted to RNase A treatment. The lysate is then incubated with a solution containing a chaotropic salt. The mixture is added to the Magnetic beads which capture the DNA in presence of the specific binding buffer. Three washing step remove cellular debris, proteins, polysaccharides and enzyme inhibitors.
DNA is finally eluted in water and ready to use. The method provides rapid, safe, simultaneous purification of many samples, eliminating the use of organic solvents and the need of DNA precipitation.

Kit contents

1X Buffer DE 1 (Lysis buffer): 40 ml
1X Buffer DE 2 (Guanidine buffer): 20 ml
1X Buffer DE 3 (Binding buffer): 40 ml
1X Buffer DE 4 (Washing buffer): 20 ml
1X Proteinase K: 10 mg
1X RNase A: 5 mg
2X Magnetic beads : 1.25 ml

Other supplies required

· Ethanol, absolute.
· Water, sterile double-distilled.
· Magnetic stand.
· Vortex.
· Disposable latex gloves.
· Precision pipettes.
· Sterile pipette tips with aerosol barrier.
· Sterile 1.5 ml tubes.
· Water bath.

Storage

Store at -20°C
Buffer DE 1, DE 2, DE 3 e DE 4 must be stored at room temperature.
Magnetic beads must be stored at 2-4°C.
RNase A and **Proteinase K** must be stored at -20°C.
Note: After dissolution, RNase A and Proteinase K solutions are stable at -20°C for 6 months

Precautions

Safety Information:

⟹ **BUFFER DE 2 contains guanidine hydrochloride: harmful, irritant**

The user should always pay attention to:

· use pipette tips with filters;
· avoid cross-contamination between samples;
· always wear gloves and lab coats;
· properly dispose of all contaminated materials;
· decontaminate work surfaces;
· use a biosafety cabinet whenever aerosols might be generated.

Procedure

The preparation of the following working solution is required:

· **Proteinase K**: dissolve 10 mg of proteinase K in 0.5 ml sterile double-distilled Water, aliquot solution and store at -20°C.
· **RNase A** dissolve 5 mg of RNase A in 0.5 ml sterile doubledistilled Water, aliquot solution and store at -20°C.
· **50% Ethanol**: mix 25 ml absolute Ethanol with 25 ml sterile double-distilled Water, store at room temperature.

1. Centrifuge the fixed phytoplanktonic sample at 3,000 *g* for 10 min at 15°C. Wash twice with artificial seawater and perform the DNA extraction on the pellet. Pellected cells could be frozen at -20°C for several weeks.

2. Add 400 μl Buffer DE 1 to the sample.

3. Add 5 μl Proteinase K solution (see above) and mix by vortexing for 20 sec.

Incubate at 50°C for 30 min.

Optional: Add 1.5 μl RNase A solution (see above)

4. Incubate at 50°C for 1 hour. Vortex every 15 minutes to facilitate the cell lysis.

5. Add 200 μl Buffer DE 2 to the sample and mix by vortexing.

Incubate at 65°C for 10 min and then vortex again.

6. During the incubation, transfer 50 μl of resuspended Magnetic beads in a 1.5 ml tube.

Immobilize the Magnetic beads on the magnetic stand and remove the supernatant.

Take the tube off the magnetic stand, add 500 μl sterile double-distilled Water and mix.

Immobilise the Magnetic beads on the magnetic stand and repeat the washing step once.

Remove the water from magnetic Magnetic beads immediately prior to use.

7. Transfer the lysate into the bead-containing tube and add 400 μl Buffer DE 3.

Incubate at room temperature for 15 min. During incubation mix frequently by inverting the tube several times or place it on a rocking platform.

Immobilise the Magnetic beads on the magnetic stand, remove and discard the supernatant.

8. Resuspend the Magnetic beads in 200 μl Buffer DE 4.

Immobilise the Magnetic beads on the magnetic stand, remove the supernatant.

9. Add 200 μl 50% Ethanol.

Incubate at room temperature for 5 min frequently mixing by inverting the tube several times.

Immobilise the Magnetic beads on the magnetic stand, remove the supernatant.

Repeat the washing step with ethanol and air-dry the Magnetic beads for 3-5 min.

10. Elute the DNA adding 50 μl double-distilled Water to the Magnetic beads-DNA complex and incubating for 10 min at 65°C frequently mixing.

Immobilise the Magnetic beads on the magnetic stand and collect the supernatant.

References

1) Marin I, Aguilera A, Reguera B, Abad JP (2001) Preparation of DNA suitable for PCR amplification from fresh or fixed single dinoflagellate cells. Biotechniques 30: 88-93.

2) Huang J, Ge X, Sun M (2000) Modified CTAB protocol using a silica matrix for isolation of plant genomic DNA. Biotechniques 28: 432-434.

3) Adachi M, Sako Y, Ishida Y (1994) Restriction fragment length polymorphism of ribosomal DNA internal transcribed spacer and 5.8 S region in Japanese *Alexandrium* species (Dinophyceae). Journal of Phycology 30: 857-863.

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