Biospin Marine Animal Genomic DNA Extraction Kit

TECHNICAL SUPPORT:

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Kit Components

Cat#	BSC27S1	BSC27M1
Components	**50Tests**	**100Tests**
FL Buffer	30 mL	60mL
PK Solution	0.5 mL	1mL
WS Buffer	5 mL	10mL
Binding Buffer	35 mL	70mL
PW Buffer	12 mL (add 18 mL ethanol before use)	24mL (add 36mL ethanol before use)
Wash Buffer	26mL (add 39 mL ethanol before use)	52mL (add 78mL ethanol before use)
Elution Buffer	10mL	20mL
Spin Column	50	100
Handbook	1 copy	1 copy

Storage

The PK solution is to be stored at 2-8℃, others at 15-25℃.
All reagents, when stored properly, are stable for 18 months.

Introduction

The kit provides a very simple, fast and economic way for the isolation of pure high–molecular-weight genomic DNA from marine animal tissues, adopting the Genomic DNA Buffer Set. The simple purification procedure, based on the remarkable selectivity of Biospin membrane, allows isolation of high yields of pure genomic DNA without proteins and other contaminants. It not requires expensive equipment, involves only few steps, and completely avoids the use of toxic and hazardous reagents such as phenol and chloroform.
The pure DNA can be applied extensively in PCR/Real-time PCR, sequencing, Southern blot, mutant analysis, SNP and the others.

Principle

At first, the animal tissue is lysed in FL Buffer; DNA in the sample is liberated under the cooperation of FL Buffer and PK solution. After centrifuging, the impurity will be discarded. Released DNA is bound exclusively and specifically to the Biospin membrane in presence of Binding Buffer under appropriate salt and pH conditions. Denatured protein and other contaminants are removed by several washing procedures. The DNA is then eluted from the membrane with the Elution Buffer.

Apparatus and Materials to Be Supplied by the User

* Sterile 2.0mL microcentrifuge tubes
* 10μL/100μL/1000μL tips
* Microcentrifuge capable of 14,000g
* Absolute ethanol

Important notes

1. Please add absolute ethanol to PW Buffer and mix thoroughly before the first use.

2. Please add absolute ethanol to Wash Binding Buffer and mix thoroughly before the first use.
3. FL Buffer may form precipitates upon storage. In case of precipitate forming, please incubate the buffer at 37℃ until the precipitate has fully dissolved.

Protocol

1. Grind the tissue into powder under the liquid nitrogen or ice bath.
2. Transfer up to 50mg tissue to a 1.5 or 2.0mL micro centrifuge tube. Note: lysis will be affected by the degree of grinded sample
3. Add 600μL FL buffer and 10μL PK solution（*Optional I :Add 2μL of 100mg/mL RNase A*）.Mix thoroughly.
4. Incubate in a bath to 56℃ to effectively complete lysis. And remove the tube from 56℃.If the sample is difficult to be lysised，please extend the incubation time. Centrifuge at 14,000*g for 3 mins to pellet debris. (**Note:**Lysis time depends on the amount and type of tissue used；average time is usually under 3 hours. One can allow lysis to proceed overnight.)
5. Transfer supernatant 500μL to a 2.0 microcentrifuge tube (*OptionalII：Add 100μL WS buffer, mix thoroughly, centrifuge at 12,000g for 3 minutes, transfer supernatant 500μL to a new 2.0 microcentrifuge tube).*
6. Add 700μL Binding Buffer and 300μL absolute ethanol, mix thoroughly.
7. Transfer the mixture to the Spin Column. Centrifuge at 10,000 x g for 1 minute. Discard flow-through. Sample volume is more than 750μl, simply load and spin again.
8. Add 500μL of the PW Buffer to the Spin Column. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through.
9. Add 600μL Wash Buffer to the Spin Column. Centrifuge at 10,000 x g for 30 seconds. Discard flow-through.
10. Repeat step 9th.
11. Centrifuge for an additional 1 minute at 10,000 x g and transfer the Spin Column to a sterile 1.5ml microcentrifuge tube.
12. Add 50μL to 100μL Elution Buffer, Incubate at room temperature for 1 minute.
13. Centrifuge at 12,000 x g for 1 minute. The buffer in the microcentrifuge tube contains the DNA.
14. The purified DNA can be used directly for kinds of downstream molecular biological experiments. Store at -20℃ if not used immediately.

FAQ

Q1: We can extract DNA from how many tissues for one test?

A: 50mg max.

Q2: Can we use homogenizer to grind samples, besides liquid nitrogen.

A: Yes.

Q3: How to wipe off the RNA included in the extracted DNA?

A: We can add RNase A to digest the RNA following with the method listed in Specification.

Q4: How long about the extracted genomic DNA fragments?

A: Length of the extracted genomic DNA fragments is about 30~50KB in general.

Q5: What can we do when DNA extraction yield is low?

A: The sample, FL Buffer and PK should be mixed thoroughly. The lysis efficiency will be affected and then lead to the low yield. Otherwise，we can extend the incubation time for 3h,even for the night.

Q6: Can we improve the DNA extraction yield using time after time elution?
A: Yes, we can adopt double times of elution (ex.: The elution liquid is 100μL, can be divided into 2 times, 50μL for each time.) Aside, we can improve the DNA yield by preheating the elution liquid.
Q7：:When can we use Optiona lII?
A：If the sample protein in high content，such as spawn，protein released from sample cell can be removed by Optiona lII. But usually, we do not advise to use Optiona lII, because this step may cause a lot of DNA loss.

Analysis DNA

⊕Absorbance analysis

Get some DNA，diluted in a advisable factor with elution buffer.

Survey the OD_{260} ，OD_{280} and OD_{320}.

Expressions：concentration（μg/mL）＝50×OD_{260}×dilution fact

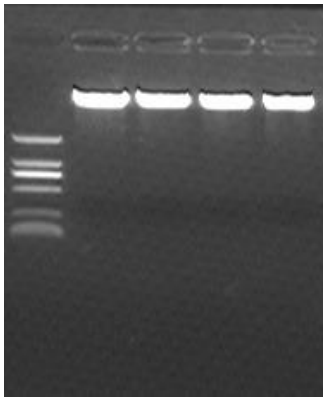
Target： $2.0 \geq OD_{260-320} / OD_{280-320} \geq 1.7$

Notice: $1.0 \geq OD_{260} \geq 0.1$, the result of ratio is much reliable.

⊕Agarose Gel Analysis

0.8～1% Agarose gel

Example 1：Fishtail



Example 2：Shell meat