SimEX™ Tissue DNA Extraction Kit

Cat. No: SBL15-1832

USER MANUAL

INTRODUCTION

SimEX™ Tissue DNA Extraction Kit is designed for isolation of DNA from animal tissues.

PRODUCT DESCRIPTION

The SimEX™ Tissue DNA Extraction Kit includes the lysis buffer, wash buffers, and elution buffer which are used for lysing the cells, elimination of the unpleased macromolecules, and eluting purified DNA, respectively. According to the use of the special columns, the SimEX™ Tissue DNA Extraction Kit provides fast and high-quality DNA for use in procedures such as Real-time PCR and sequencing.

KIT CONTENTS

Components	Labels	Volume
Proteinase K	Proteinase K	20 mg
Proteinase K Dilution Buffer	PKDB	1 ml
Lysis Buffer	SLB	10 ml
Binding Buffer	SBB	10 ml
Wash Buffer 1	SWB1	15 ml
Wash Buffer 2	SWB2	12 ml
Elution Buffer	SEB	10 ml
Collection tube	Collection tube	50
Columns	Columns	50

Additional Required Materials

1. Absolute ethanol
2. Table-top micro centrifuge, 10,000 xg (13,000 rpm)
3. Thermal block or water bath
4. Vortex mixer
5. 1.5 ml tube (for preparation of lysate)

Before use

- Dissolve proteinase K in 1ml Proteinase K Dilution Buffer (PKDB)
- Add the correct amount of absolute ethanol to solution SWB1 and SWB2
- Preheat the solution SEB to 57˚C.

PROTOCOL; Isolation of DNA from animal tissues

a) Cell lysis

1. Centrifuge the solution containing tissue to precipitate tissue particles if the tissue in the solution had reached you; then discard amounts of the supernatant until 200µl remain on the precipitant. Otherwise place 20-30 mg tissue in the microfuge tube.
2. Add 20µl of Proteinase K.
3. Add 200µl of Lysis buffer (SLB) to the sample and mix immediately by vortex mixer.
4. Incubate at 55℃ for 30 min.
5. Add 200µl of Binding buffer (SBB) to the sample and mix immediately by Pipetting.
6. Incubate at RT for 5 min.

b) Removing contaminations

7. Add 200µl of Ethanol 96% and mix well by pipetting.

Do not vortex, this might reduce DNA yield!!

8. Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a 2 ml tube).
9. Close the tube and centrifuge at 13,000 rpm for 1 min.
10. Discard collected solution in the collection tube and add 500µl of Washing buffer 1 (SWB1) and centrifuge at 13,000 rpm for 1 min.
11. Pour the solution from the 2 ml tube into a disposal bottle.
12. Carefully add 700µl of Washing buffer 2 (SWB2) and centrifuge at 13,000 rpm for 1 min.
13. Centrifuge once more at 13,000 rpm for 1 min to completely remove ethanol.

c) Elution

14. Transfer the Binding column tube to a new 1.5 ml tube for elution (supplied), add 200µl of Elution buffer (SEB, or nuclease-free water) onto column tube, and wait for at least 1 min at RT (15~25℃) until SEB is completely absorbed into the glass fiber of column tube.
15. Centrifuge at 13,000 rpm for 1 min to elute.

About 180µl ~ 200µl of eluent can be obtained when using 200µl of Elution buffer. The eluted genomic DNA is stable and can be used directly, or stored at 4℃ for later analysis. For long-term DNA storage, you should elute with Elution buffer (SEB) and store at -20˚C, because DNA stored in water is subject to acid hydrolysis. About 6µg of DNA in 200µl of eluent (30 ng/µl) with an A260/A280 ratio of 1.6 ~ 1.9.

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20 µl of Proteinase K
200 µl Lysis Buffer (SLB)
Tissue Sample
(<= 25 mg)

Incubate 55°C / 30 min

Centrifuge the solution containing tissue to precipitate tissue particles; then discard amounts of the supernatant until 200µl remain on the precipitant.

200 µl BInding Buffer (SBB)
mix well by pipeting
incubate at RT for 5 min

Add 200 µl Ethanol 96%

Transfer to the filtered column

Centrifuge 13.000 rpm 1 min

Discard collected solution in the collection tube

500 µl SWB1

Centrifuge 13.000 rpm 1 min

Discard collected solution in the collection tube

700 µl SWB2

Centrifuge 13.000 rpm 1 min

Discard collected solution in the collection tube

Centrifuge Empty column to throw away remained

Transfer the filtered column to the new 1.5 ml tube

Add Preheated Elution Buffer (SEB) (100 µl -200 µl)

Centrifuge 13.000 rpm 1 min

Store collected solution at -20 °C