SimEX™ Blood DNA Extraction Kit

Cat. No: SBL15-2005/50

USER MANUAL

INTRODUCTION

SimEX™ Blood DNA Extraction Kit is designed for isolation of DNA from white blood cells, tissue culture cells and animal tissue.

PRODUCT DESCRIPTION

SimEX™ 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, SimEX™ 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	SBL	11 ml
Wash Buffer 1	SBW1	25 ml
Wash Buffer 2	SBW2	52 ml
Elution Buffer	SBE	10 ml
Columns	Columns	50
Collection Tube	Collection tube	100

Additional Required Materials

1. Absolute ethanol
2. Table-top microcentrifuge, 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)
- Preheat the solution SBE to 57˚C.

PROTOCOL; Isolation of DNA from whole blood, buffy coat, and cultured cells.

a) Cell lysis

1. Add 20µl of Proteinase K to a clean 1.5ml tube.
2. Apply 200µl of whole blood, buffy coat or 10^4~10^8 cultured cells to the tube containing proteinase K.
3. Add 200µl of Lysis buffer (SBL) to the sample and mix immediately by vortex mixer.
4. Incubate at 57℃ for 20 min.

b) Removing contaminations

5. Add 200µl of Ethanol 96% and mix well by pipetting. *Do not vortex, this might reduce DNA yield!!*
6. Carefully transfer the lysate into the upper reservoir of the Binding column tube (fit in a 2 ml tube).
7. Close the tube and centrifuge at 13,000 rpm for 1 min.
8. Discard collected solution in the collection tube and add 500µl of Washing buffer 1 (SBW1) and centrifuge at 13,000 rpm for 1 min.
9. Pour the solution from the 2 ml tube into a disposal bottle.
10. Carefully add 700µl of Washing buffer 2 (SBW2) and centrifuge at 13,000 rpm for 1 min.
    Optional: The second wash step (SWB2) could be repeated twice in which 500 µl of SWB2 is added each time.
11. Centrifuge once more at 13,000 rpm for 1 min to completely remove ethanol.

c) Elution

12. Transfer the Binding column tube to a new 1.5 ml tube for elution (supplied), add 200µl of Elution buffer (SBE, or nuclease-free water) onto column tube, and wait for at least 1 min at RT (15~25℃) until SBE is completely absorbed into the glass fiber of column tube.
13. 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 (SBE) and store at -20˚C, because DNA stored in water is subject to acid hydrolysis. About 12µg of DNA in 200µl of eluent (60 ng/µl) with an A260/A280 ratio of 1.6 ~ 1.9 can be typically obtained from 200µl of whole blood (~ 5 X 10^6 leukocytes/ml).

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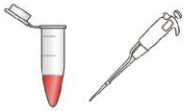


20 µl of Proteinase K
200 µl Lysis Buffer (SBL)
200 µl Sample

Vortex 15 Sec

Incubate 57°C / 20 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 SBW1

Centrifuge 13.000 rpm 1 min

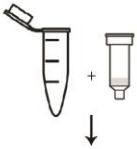
Discard collected solution in the collection tube

700 µl SBW2

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 (SBE) (100 µl -200 µl)

Centrifuge 13.000 rpm 1 min

Store collected solution at -20°C