SimEX™ DNA Gel Extraction kit

Cat. No: SBL15-1615

USER MANUAL

INTRODUCTION

SimEX™ DNA Gel Extraction kit is designed for isolation of DNA from agarose gel.

KIT CONTENTS

Components	Labels	Volume
Gel Dissolving Buffer	GDB	40 ml
DNA Wash Buffer	DWB	50 ml
DNA Elution Buffer	DEB	5 ml
Columns	Columns	50

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

- Add the correct amount of absolute ethanol to DWB solution.
- Preheat the solution DEB to 55˚C.

PROTOCOL

DNA fragments are excised from an agarose gel and are diluted by addition of 2-3 volumes of Gel Dissolving Buffer. For a typical 100 mg (100µl) gel slice, 200-300µl of Gel Dissolving Buffer is added.
All centrifugation steps should be carried out at 12,000 rpm in a standard laboratory microcentrifuge at room temperature.

1. Cut the DNA fragment to be purified from the agarose gel. Use care to trim excess agarose. Transfer it to a 1.5 ml microcentrifuge tube and weigh the gel slice.

Note: *Using UV light to visualize the slice is common, but exposure time should be kept as short as possible to minimize damage to the DNA. Use long-wave UV when possible, as shorter wavelengths induce greater damage. Also, trim off excess agarose from the perimeter of the band to minimize the amount of dissolving buffer needed, and to reduce the time necessary to extract the DNA.*

2. Add 2-3 volumes of Gel Dissolving Buffer to the tube with the slice.

Note: *If the volume of the dissolved sample exceeds 800µl, the loading of the sample onto the column should be performed in multiple rounds to not exceed the volume constraints of the spin column.*

3. Incubate the sample at 55°C, vortexing periodically until the gel slice is completely dissolved (generally 10–15 minutes).

Note: *Failure to dissolve all the agarose will decrease the recovery yield due to incomplete extraction of the DNA and potential clogging of the column by particles of agarose.*

4. Insert the column into collection tube and load 800µl sample onto the column. Spin for 1 minute, then discard flow-through.

Repeat step 4 for the not centrifuged sample if the amount of solution was greater than the capacity of the column in the step 2.

5. Re-insert column into collection tube. Add 750µl DNA Wash Buffer and spin for 1 minute; then discard flow-through.
6. Repeat step 5 with the empty column to ensure that the filter is dry.
7. Transfer column to a clean 1.5 ml microfuge tube. Use care to ensure that the tip of the column has not come into contact with the flow-through. If in doubt, respin for 1 minute before placing into clean microfuge tube.
8. Add 40 µl of DNA Elution Buffer to the center of the matrix. Wait for 5-10 minute, and spin for 1 minute to elute DNA.
9. Repass flow-through from the column.

Heating the elution buffer to 55°C prior to use can improve yield. Care should be used to ensure the elution buffer is delivered onto the matrix to maximize elution efficiency.

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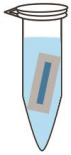


1. up to 300 mg Gel slice
2. 2-3 Vol GDB Buffer

3. 55°C for 15 min
vortexing periodically

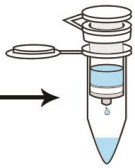
4. load 800 µl sample
onto the column

Spin 1 min
discard flow-through

5. add 750 µl DWB

Spin 1 min
discard flow-through

6. Spin Eempty column 1 min



7. Transfer Column

8. add 40 µl DEB
Wait for 5-10 minute

9.Repass
flow-through
from the
column

Spin 1 min

Repeat step 4 for the not centrifuged sample if the amount of solution was greater than the capacity of the column in the step 2