

SeqPrep™ 96 Plasmid Prep Kit

Product	Catalog #	Purifications
SeqPrep™ 96 Plasmid Prep Kit (2 Plates)	25659	192
SeqPrep™ 96 Plasmid Prep Kit (10 Plates)	84359	960

Description

The SeqPrep™ 96 Plasmid Prep Kit is a system that provides a novel technology for the isolation of high copy plasmid DNA from bacterial cell cultures. The SeqPrep™ 96 Plate has been surface-modified to enable efficient and highly selective DNA binding to the surface of the wells. DNA binds to the surface while cells are lysed. The plate can be used for culture growth, DNA purification and DNA storage.

Key Features

- 10-15 minute processing time.
- Average DNA yield of 1.0µg per well.
- DNA is suitable for routine molecular biological applications such as DNA sequencing, PCR, and restriction analysis.
- No sample transfers are needed during the process.
- Excellent for both manual operation and automation.

Kit Components	25659	84359
SeqPrep™ 96 Plates	2 plates (1 x PN 4050218)	10 plates (2 x PN 4050219)
SeqPrep™ 96 Lysis Solution	25 ml (1 x PN 4050222)	125 ml (1 x PN 4050223)
Enzyme Mix	0.25 ml (1 x PN 4050220)	1.25 ml (1 x PN 4050221)
Wash Solution	7 ml (1 x PN 4050225)	36 ml (1 x PN 4050226)
Gas-Permeable Plate Sealers	2 seals (1 x PN 4050229)	10 seals (1 x PN 4050230)

Warning: This product is intended for **research use only**. It is not to be used for diagnostic purposes in humans or animals.

Storage and Stability Conditions

Store the Enzyme Mix at -20°C.

Store the remaining items at room temperature.

After addition of Enzyme Mix to SeqPrep™ 96 Lysis Solution, store the mixture at 4°C.

Lysis Solution/Enzyme Mix is stable at 4°C for one month.

Equipment and Materials Required

1. Multichannel pipettor / dispenser
2. Isopropanol
3. De-ionized water (dH₂O) or 10mM Tris-HCl, pH8.0
4. Centrifuge with microplate carriers capable of reaching 850 x g
5. Vortexer with a microplate adaptor, Titer plate shaker, or **Eppendorf® MixMate™** (Part# 5353 000 01 4)
6. Adhesive Plate Sealers (Edge BioSystems, Cat. # 48461)

Quality Control

Tested for functionality in DNA sequencing with 1/8th reactions.

Before starting

Bacterial Cultures

1. In the SeqPrep 96 Plate, inoculate 200 µl of 2xYT containing the appropriate antibiotic with a single colony or 5 µl of glycerol stock. Cover with a gas-permeable seal. Incubate at 37 °C while shaking at 300 rpm for 12-18 hrs.

Note:

- It is recommended to start with 200 µl/well 2xYT.
- Bacterial cells grown in LB will result in lower DNA yield. Bacterial cells grown in TB may result in relatively higher contamination.
- Bacterial cell growth in the SeqPrep™ 96 Plate is similar to the growth in a culture tube. Bacteria will grow better in the SeqPrep™ 96 Plate than they will in a 96-well culture block.

- If you witness a loss of more than 50 µl of culture after overnight growth, use a non-gas-permeable plate sealer to cover the plate. Using a syringe needle punch two holes per well to allow for sufficient aeration.
- If a 96-well culture block is used for the culture, inoculate 0.5 – 1 ml of 2xYT containing the appropriate antibiotic with a single colony or with 5 µl of glycerol stock. Cover with a gas-permeable seal. Incubate at 37°C while shaking at 300 rpm for 12-18 hrs. Pellet cells at 1500 × g for 10 min. Decant the supernatant and invert the plate on an absorbent pad. Tap gently to ensure all of the supernatant has been removed. Add 50 µl of TE buffer and re-suspend the cells thoroughly by shaking rapidly for 1 min. Transfer 20 µl of the re-suspended cells to the SeqPrep™ 96 Plate and proceed directly to cell lysis (step 4 of the protocol).
- If the cells are grown in 2xYT in a culture tube or larger vessel, transfer 200 µl of the bacterial culture to the wells of a SeqPrep™ 96 Plate.

Prepare the Lysis Solution/Enzyme Mix

1. Thaw the Enzyme Mix completely and thoroughly vortex to mix. If necessary, spin the tube briefly to collect all of the liquid in the bottom of the tube.
2. Transfer the entire content of Enzyme Mix tube to the bottle of Lysis Solution. This will be referred to as Lysis Solution/Enzyme Mix. Mix the bottle thoroughly and write “/Enzyme Mix” following Lysis Solution on the label.

Volume of Lysis Solution	Volume of Enzyme Mix
25 ml	0.25 ml
125 ml	1.25 ml

3. Place the bottle on ice before and during use. Store any unused Lysis Solution at 4°C for up to one month.

Note:

- It may be necessary to just make enough Lysis Solution/Enzyme Mix for the day’s use if it is not certain that the entire Lysis Solution/Enzyme Mix will be used within a month. To do so, mix 1 volume of Enzyme Mix to 100 volumes of Lysis Solution to make a volume ratio of 1:100. For example, add 120 µl of Enzyme Mix to 12 ml of Lysis Solution to make enough Lysis Solution/Enzyme Mix for processing 96 samples on a SeqPrep™ plate.
- Repeated freezing and thawing decreases the activity of the Enzyme Mix. If a 10-plate kit or 50-

plate kit is purchased, it is recommended to aliquot the Enzyme Mix based on the frequency of DNA preparation when it is thawed for the first time. For example, a 240 µl fraction of Enzyme Mix can be aliquoted if two plates are processed at a time or expected to be processed every month.

Prepare Wash Solution

1. Use isopropanol only. The use of ethanol will result in significant loss in yield.
2. Add the appropriate volume of 100% isopropanol to the entire bottle of concentrated Wash Solution as indicated on the label; this will be referred to as “prepared Wash Solution”. Mix the bottle thoroughly and write “Isopropanol Added” on the label. Keep the bottle lid tightly closed to prevent alcohol evaporation.

Volume of Wash Solution	Volume of Isopropanol
7 ml	16 ml
36 ml	84 ml

Note:

- A different volume of Wash Solution can also be prepared by mixing 7 volumes of isopropanol and 3 volumes of concentrated Wash Solution to make a volume ratio of 7:3. For example, add 7 ml of isopropanol to 3 ml of concentrated Wash Solution (concentrate).

Prepare 70% Isopropanol

1. The 70% isopropanol can be prepared by mixing 7 volumes of 100% isopropanol and 3 volumes of water to make a volume ratio of 7:3. For example, add 70 ml of isopropanol to 30 ml of water. Keep the bottle lid tightly closed to prevent alcohol evaporation.

Recommended Protocol

- 1. Centrifuge the SeqPrep™ 96 Plate at 850 x g for 3 minutes.**
 - Do not over spin; excessive centrifugation will cause difficulty in resuspending cells in the lysis solution.
 - For determination of RPM from RCF, see technical information on page 4 or visit our website at www.edgebio.com and click on Technical Support.
- 2. Immediately remove supernatant.**
 - Take off the gas-permeable seal and decant the supernatant by inverting the plate. Gently blot the inverted plate on a paper towel or an absorbent pad; **do not tap on the plate.** There should be about 5 µl to 15 µl of media left in each well.
- 3. Cover with an adhesive plate sealer and vortex vigorously for 30 seconds to resuspend cells in remaining media.**
 - If using the Eppendorf® MixMate™ vortex at 1650 RPM for 30 seconds.
 - Alternatively, if using another vortex model, secure the plate to the vortex mixer and gradually increase the speed to a vigorous agitation while avoiding splashing of the liquid to the top of the plate.
 - Cell pellets should be fully resuspended before addition of the lysis solution. This will ensure complete lysis and higher DNA quality.
- 4. Add 100 µl of Lysis Solution/Enzyme Mix to the resuspended cells.**
- 5. Vortex or shake for 3-5 minutes to lyse the cells.**
 - If using the Eppendorf® MixMate™ vortex at 1000 RPM for 3-5 minutes.
- 6. Remove the lysate by decanting and blot the plate.**
 - Blot the plate vigorously onto an absorbent pad or a paper towel several times. Plasmid DNA is now bound to the surface of the wells and will not be released by blotting.
 - Alternatively, if you see any cell debris, turn the plate face down onto a paper towel or an absorbent pad and invert spin the plate at 850 x g in a centrifuge for 1 minute.
- 7. Add 100 µl of prepared Wash Solution to the samples.**
- 8. Vortex or shake for 30 seconds.**
 - If using the Eppendorf® MixMate™ vortex at 1000 RPM for 30 seconds.
- 9. Remove the wash by decanting and blot the plate.**
 - Blot the plate vigorously onto an absorbent pad or a paper towel several times to get as much wash solution out as possible.
- 10. Add 100 µl of 70% Isopropanol to the samples.**
 - Use isopropanol only for the washes. The use of ethanol will result in significant loss in yield.
- 11. Vortex or shake for 30 seconds.**
 - If using the Eppendorf® MixMate™ vortex at 1000 RPM for 30 seconds.
- 12. Remove the 70% Isopropanol by decanting, and blot the plate.**
- 13. Repeat Steps 10 through 12 once.**
- 14. Invert spin the plate at 850 x g for 1 minute.**
 - Turn the plate face down onto a paper towel or an absorbent pad, and place in the centrifuge to spin.
- 15. Air dry the plate at room temperature for 30 minutes.**
 - Leave the plate face up on the bench.
 - Do not leave for an extended period longer than 2 hours as this will cause the plasmid DNA to bind too tightly to the plate.
 - Make sure that the plate is completely dry from isopropanol before eluting the DNA in the final step. Remnant isopropanol can affect the quality of the DNA.
- 16. Add 40 µl of 10 mM Tris•HCl, pH 8.0. Vortex or shake for 1 minute, or incubate at room temperature for 5 minutes.**
 - If using the Eppendorf® MixMate™ vortex at 1000 RPM for 1 minute.
 - Water can be used for elution, but may cause the DNA band to smear on an agarose gel. However, the smearing has no evident effect on downstream applications.
- 17. DNA is ready for immediate use.**
 - Use PicoGreen assay or agarose gel electrophoresis for DNA quantification instead of OD₂₆₀. The OD is usually over-inflated when using OD₂₆₀ for quantification.
 - DNA may be stored in the SeqPrep™ plate or transferred to a receiver plate for storage. To avoid evaporation properly seal the SeqPrep™ plate when storing DNA.

Technical Information

Media Preparation

2xYT (1 Liter)

16 g Bacto Tryptone

10 g Bacto Yeast Extract

5 g NaCl

Add dH₂O to one liter and autoclave

Luria Bertani (1 Liter)

10 g Bacto Tryptone

5g Bacto Yeast Extract

10 g NaCl

Add dH₂O to one liter and autoclave

Terrific Broth (1 Liter)

12 g Bacto Tryptone

24 g Bacto Yeast Extract

4 ml Glycerol

2.2 g KH₂PO₄

9.4 g K₂HPO₄

Add dH₂O to one liter and autoclave

Converting RPM to RCF

An accurate determination of the centrifugation speed is very important. The relative centrifugal force (RCF) specified in the protocol is converted to revolutions per minute (RPM) using the following formula:

$$RCF = 1.12 r \left(\frac{RPM}{1000} \right)^2$$

The radius, *r*, is equal to the distance in millimeters between the axis of rotation and the plate carrier in the centrifuge bucket.

After measuring the radius for the specific centrifuge and accessories to be used, the proper RPM setting is calculated as follows:

$$RPM = 1000 \sqrt{\frac{RCF}{1.12 r}}$$

To achieve RCF = 850 x g:

$$RPM = 27,549 \sqrt{\frac{1}{r}}$$

Note:

- A microplate carrier is needed for centrifugation. The centrifugation force of 850 x g is achieved at about 2200 rpm with a GH3.8 or GH3.8A rotor in a Beckman Coulter centrifuge or with a H1000-B rotor in a Sorvall centrifuge.