SeqPrep™ 96 HP Plasmid Prep Kit

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

Description

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

Key Features

- Suitable for high copy number plasmids, fosmids
- 20-25 minute processing time
- No plate transfer steps
- DNA yield – average up to 1 µg per well
- Provides high quality DNA sequencing results
- Convenient for manual operation
- Automatable

Kit Components	25659	84359
SeqPrep™ 96 HP Plates	2 plates	10 plates
Gas-Permeable Plate Sealers	2 sealers	10 sealers
Enzyme Mix	0.25 ml	1.25 ml
HP Lysis Solution	25 ml	125 ml
HP Wash Solution I	40 ml	175 ml
HP Wash Solution II	12 ml	70 ml
HP Elution Buffer	10 ml	50 ml
TB Medium	45 ml	Not included

Storage and Stability

Store the Enzyme Mix at -20°C.

Store the remaining items at room temperature.

After addition of Enzyme Mix to HP 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. Centrifuge with microplate carriers capable of reaching 850 × g
2. Laboratory microplate shaker capable of operating at 1000 rpm (*e.g.,* Lab-Line Titer Plate Shaker, Thermo Cat# 14-271-9; or Eppendorf® MixMate™, Part# 5353 000 01 4)
   - A vortexer with a microplate adaptor is not recommended due to the vibration.
3. Multi-channel pipettor
4. 96-100% ethanol
5. Adhesive Plate Sealers (EdgeBio Cat# 48461)
6. Terrific Broth (TB) Medium for catalog number 84359

Quality Control

Plasmid pUC19 is purified and sequenced for functionality test.

Before starting

Bacterial Culture

- The use of Terrific Broth (TB) medium for bacterial growth is important as it will typically give the best yield. The glycerol and phosphates in TB medium ensure sufficient yield for downstream applications. *See TB Media recipe in Technical Information.*

- For first time use of the kit, pick several wells to measure the OD_{600}, the typical OD_{600} will be between 3.0 and 5.0.

Prepare HP Lysis Solution/Enzyme Mix

1. Thaw Enzyme Mix completely and 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 the 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.

3. Place Lysis Solution/Enzyme Mix on ice before use.

4. Store any unused Lysis Solution/Enzyme Mix at 4°C for up to one month.

5. Mix well by inverting the bottle before use.

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. 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™ HP plate.

- Repeated freezing and thawing decreases enzyme activity. It is recommended to aliquot the Enzyme Mix based on the frequency of DNA preparation. For example, make aliquots of 240 µl of Enzyme Mix if two plates are processed at a time or expected to be processed every month.

Prepare HP Wash Solution II

Add 4 volumes of 96-100% ethanol to 1 volume of concentrated HP Wash Solution II as indicated on the label of the HP Wash Solution II bottle. Mark 'ethanol added'. Mix thoroughly. Keep the bottle tightly closed to prevent alcohol evaporation.

Volume of HP Wash Solution II	Volume of 96-100% ethanol
12 ml	48 ml
70 ml	280 ml

Automation of the procedure

1. For automated operation, load the plate on a biorobot after complete cell resuspension (step 5).

2. Use pipette tip-mixing in all the purification steps.

3. Wash twice with HP Wash Buffer II.

4. After wash steps, invert-spin the plate, air-dry on the deck and elute as described in steps 12-15.

Note:

It is critical to mix thoroughly in each step and aspirate to remove all the liquid after mixing.

Recommended Protocol

1. **In the SeqPrep™ 96 HP Plate, inoculate 200 µl of TB medium containing appropriate antibiotic with a fresh single colony or 3 µl of glycerol stock for each well. Seal with a gas-permeable sealer.**
   - For fosmids, use 10 µl of glycerol stock or un-induced pre-culture to inoculate medium containing the appropriate inducer.
2. **Incubate at 37°C while shaking at 300 rpm for 19 hrs.**
3. **Centrifuge the SeqPrep™ 96 HP Plate at 850 x g for 3 min.**
   - For determination of RPM from RCF, see technical information on page 4 or Technical Support on www.edgebio.com.
4. **Peel off the sealer. Decant the supernatant. There should be about 5 µl to 15 µl of media left in each well for cell resuspension.**
5. **Shake the plate vigorously on a microplate shaker at approx. 1000-1500 rpm for 2 min to fully resuspend cells in remaining media.**
   - Full resuspension of cell pellets before adding the lysis solution is critical for complete lysis.
   - If the pellet failed to resuspend, use pipette to dislodge and disperse the cells.
   - Use a laboratory microplate shaker for thorough and steady mixing. It is not necessary to seal the plate with an adhesive plate sealer in this and subsequent steps, unless there is severe splashing that could cause cross contamination.
   - A vortexer with microplate adaptor is not recommended. If it is used, control the speed carefully. Seal the plate with an adhesive plate sealer to avoid contamination.
6. **Add 100 µl of ice-cold Lysis Solution/Enzyme Mix. Mix by shaking at approx. 1000 rpm for 5 min to lyse the cells.**
7. **Decant the lysate.**
   - Blot the plate vigorously onto a clean absorbent towel several times to remove as much excess liquid as possible.
8. **Add 150 µl of HP Wash Solution I. Shake the plate at approx. 1000 rpm for 1 min.**
9. **Decant the wash.**
   - Blot the plate vigorously onto a clean absorbent towel several times to remove as much excess liquid as possible.
10. **Add 150 µl of HP Wash Solution II. Shake the plate at approx. 1000 rpm for 1 min.**
11. **Decant the wash.**
    - Blot the plate vigorously onto an absorbent towel several times to remove as much excess liquid as possible.
12. **Invert-spin the plate at 850 x g for 1 min at room temperature with a clean absorbent pad underneath to absorb the liquid from the plate.**
13. **Air-dry the plate at room temperature for about 5 min to ensure complete evaporation of ethanol.**
14. **Add 40 µl of HP Elution Buffer. Shake the plate at approx. 1000 rpm for 1 min.**
15. **Seal the plate with an adhesive plate sealer and store.**
    - The samples are stable at 4°C for one month. Store at -20°C for long term storage. Avoid repetitive freezing and thawing.
    - Use 5 µl of sample for agarose gel analysis to estimate DNA yield.
    - A_{260} is not recommended for determination of DNA concentration for SeqPrep™ samples because of the presence of some degraded RNA. RNA will not interfere with sequencing. RNA can be eliminated by including 10 µg/ml of DNase-free RNase A in elution buffer.
    - For DNA sequencing, use 3 µl of sample regardless of concentration in a 10 µl reaction.

Technical Information

Technical Support

Visit www.edgebio.com for FAQ and Trouble Shooting Guides and technical support.

Media

Terrific Broth (TB) medium typically gives the best yield. Glycerol and phosphates in TB medium are critical for the yield. If cells overgrow in TB and die after overnight culturing, LB or 2×YT may be used instead, however glycerol and phosphates need to be added into the medium for a good yield.

- **Terrific Broth (TB)**, one liter

  12 g Tryptone
  24 g Yeast extract
  4 ml Glycerol

Adjust volume to 900 ml and autoclave
Add 100 ml of filter sterilized solution of 0.17 M KH_2PO_4 (2.3 g) and 0.72 M K_2HPO_4 (12.5 g)

- **2×YT *plus***, one liter

  16 g Tryptone
  10 g Yeast extract
  5 g NaCl
  4 ml Glycerol

Adjust volume to 900 ml and autoclave
Add 100 ml of filter sterilized solution of 0.17 M KH_2PO_4 (2.3 g) and 0.72 M K_2HPO_4 (12.5 g)

- **Luria Bertani (LB) *plus***, one liter

  10 g Tryptone
  5 g Yeast extract
  10 g NaCl
  4 ml Glycerol

Adjust volume to 900 ml and autoclave
Add 100 ml of filter sterilized solution of 0.17 M KH_2PO_4 (2.3 g) and 0.72 M K_2HPO_4 (12.5 g)

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***:

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

Examples:
The centrifugation force of 850 x g is achieved at approximately 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.