

FosPrep™ 96 Fosmid Prep Kit

Product	Catalog #	Purifications
FosPrep™ 96 Fosmid Prep Kit (2 Plates)	71393	192

Description

The FosPrep™ 96 Fosmid Prep Kit is a 96-well plate kit that provides a novel technology for high-throughput isolation of inducible fosmid DNA from bacterial cultures. The kit is designed for easy and rapid preparation of inducible fosmids for use in DNA sequencing and restriction mapping.

The SeqPrep™ 96 Plate has been surface modified to enable efficient and highly selective DNA binding to the surface of the wells. The plate can be used for culture growth, DNA purification and DNA storage. No magnets, vacuum or plate transfers are required. The process is not only highly automatable, but, simple and easy to use in manual operation.

Bacteria are grown under induction conditions in 0.016 – 0.275 ml of medium in the SeqPrep™ 96 Plate, pelleted by centrifugation (optional), and resuspended in a proprietary lysis/DNA-binding solution. DNA binds to the modified surface of the wells during lysis. The impurities are removed with a Wash Solution and 70% Isopropanol. After drying, fosmid DNA is resuspended in 10mM Tris-HCl, pH 8.0 or dH₂O. The DNA may be stored on the plate at 4°C for one month or at -20°C for long-term storage.

Kit Components	71393
SeqPrep™ 96 Plates	2 plates (1 x PN 4050218)
FosPrep™96 Lysis Solution	25 ml (1 x PN 4050228)
Enzyme Mix	0.25 ml (1 x PN 4050220)
Wash Solution	7 ml (1 x PN 4050225)
Gas-Permeable Plate Sealers	2 seals (1 x PN 4050229)

Storage and Stability Conditions

Store the Enzyme Mix at -20°C.

Store the remaining items at room temperature.

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

Lysis Solution/Enzyme Mix is stable for two weeks.

Equipment and Materials Required

1. Multichannel pipettor / dispenser
2. Isopropanol
3. De-ionized water (dH₂O) or 10mM Tris-HCl, pH 8.0
4. Centrifuge with microplate carriers capable of reaching 2500 rpm
5. Vortexer with a microplate adaptor
6. Adhesive Plate Sealers (Edge BioSystems, Cat. # 48461)
7. Gas-Permeable Plate Sealers (Edge BioSystems Cat. # 97584)

Quality Control

Tested for functionality in DNA sequencing with 1/16th reactions.

Before starting

Prepare Lysis Solution/Enzyme Mix

Add 0.25 ml of Enzyme Mix to 25 ml of FosPrep™ 96 Lysis Solution. Mix well.

Store unused Lysis Solution/Enzyme Mix at 4°C.

Prepare Wash Solution

Add 16 ml of Isopropanol to 7 ml of Wash Solution and mix. Label "Isopropanol added".

Prepare 70% Isopropanol

Mix 30 ml of dH₂O with 70 ml of Isopropanol to make 70% Isopropanol.

Recommended Protocol – High-volume Cultures

1. Grow un-induced culture in 2xYT for 6 – 16 hours.
2. In a SeqPrep™ 96 Plate, inoculate 200 µl of Terrific Broth or 2xYT with 10 µl of un-induced culture and your recommended inducer. Cover with a Gas-Permeable Plate Sealer. Incubate at 37°C and shake at 300 rpm for 12-16 hours.

OR

In a SeqPrep™ 96 Plate, inoculate 275 µl of Terrific Broth with 25 µl of un-induced culture and your recommended inducer. Cover with a plate lid. Incubate at 37°C for 16-20 hours without shaking.

3. Centrifuge the SeqPrep™ 96 Plate at 2500 rpm for 3 minutes.
4. Remove supernatant.
 - Immediately, remove cover and decant the supernatant by inverting the plate. Blot the inverted plate on a paper towel or an absorbent pad. Generally, there are about 5 µl to 15 µl of media left in the well.
5. Cover with an adhesive sealer and vortex vigorously for 20 seconds to resuspend cells in remaining media.
 - Secure the plate to a vortex mixer and gradually increase the vortex speed to obtain a vigorous agitation without splashing liquid from the wells.
6. Add 100 µl of prepared Lysis Solution/Enzyme Mix.
7. Vortex or shake for 2 minutes.

OR

Pipet to mix 8-10 times.

8. Incubate at room temperature for 3 minutes.
9. Remove lysate by decanting or pipetting.
 - Remove as much lysate as possible.
10. Wash the sample once with 100 µl of prepared Wash Solution.
 - Add 100 µl of Wash Solution. Mix by gently shaking or pipetting. Remove solution by decanting or pipetting.
11. Wash the sample twice with 100 µl of 70% Isopropanol.
 - Add 100 µl of 70% Isopropanol. Mix by gently shaking or pipetting. Remove solution by decanting or pipetting.

12. Invert the plate onto a paper towel or an absorbent pad and centrifuge at 2000 rpm for 1 minute.
 - It is important to remove as much of the remaining liquid from the last wash as possible before drying the plate. Alternatively, remove the remaining liquid slowly by pipetting. Dry the plate for at least an hour before DNA elution (Step 14).
13. Dry plate for 30 minutes at room temperature.
14. Add 30 µl of 10mM Tris-HCl, pH 8.0 or dH₂O. Seal the plate with an adhesive sealer and incubate for 1 hour at room temperature.
 - Optional: Incubate at 60°C for 20 minutes.
15. DNA is ready for immediate use.

Recommended Protocol – Low-volume Cultures

1. Grow un-induced culture in 2xYT for 6 – 16 hours.
2. In a SeqPrep™ 96 Plate, inoculate 16 µl of Terrific Broth with 2 µl of un-induced culture and your recommended inducer. Cover with an adhesive sealer. Incubate at 37°C for 16-20 hours without shaking.

3. Add 75 µl of prepared Lysis Solution/Enzyme Mix
4. Vortex or shake for 2 minutes.

OR

Pipet to mix 8-10 times.

5. Incubate at room temperature for 3 minutes
6. Remove lysate by decanting or pipetting.
 - Remove as much lysate as possible.
7. Wash the sample once with 100 µl of prepared Wash Solution.
 - Add 100 µl of Wash Solution. Mix by gently shaking or pipetting. Remove solution by decanting or pipetting.
8. Wash the sample twice with 100 µl of 70% Isopropanol.
 - Add 100 µl of 70% Isopropanol. Mix by gently shaking or pipetting. Remove solution by decanting or pipetting.

9. **Invert the plate onto a paper towel and centrifuge at 2000 rpm for 1 minute.**
 - It is important to remove as much of the remaining liquid from the last wash as possible before drying the plate. Alternatively, remove the remaining liquid slowly by pipetting. Dry the plate for at least an hour before DNA elution (**Step 11**).
10. **Dry plate for 30 minutes at room temperature.**
11. **Add 30 μ l of 10mM Tris-HCl, pH 8.0 or dH₂O. Seal the plate with an adhesive sealer and incubate for 1 hour at room temperature.**
 - Optional: Incubate at 60°C for 20 minutes.
12. **DNA is ready for immediate use.**