

FosPrep™ 384 Fosmid Prep Kit

Product	Catalog #	Purifications
FosPrep™ 384 Plasmid Prep Kit (2 Plates)	75116	768

Description

The FosPrep™ 384 Fosmid Prep Kit is a 384-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™ 384 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 0.070 ml of medium in the SeqPrep™ 384 Plate, pelleted by centrifugation, 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	75116
SeqPrep™ 384 Plates	2 plates (1 x PN 4050235)
FosPrep™ 384 Lysis Solution	30 ml (1 x PN 4050227)
Enzyme Mix	0.40 ml (1 x PN 4050211)
Wash Solution	12 ml (1 x PN 4050212)
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™ 384 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) and 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 1/24th Big Dye® v3.1 sequencing reactions.

Before starting

Prepare Lysis Solution/Enzyme Mix

Add 0.30 ml of Enzyme Mix to 30 ml of FosPrep™ 384 Lysis Solution. Mix well.

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

Prepare Wash Solution

Add 28 ml of Isopropanol to 12 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

1. **Grow un-induced culture in 2xYT.**
 2. **In a SeqPrep™ 384 Plate, inoculate 65 µl of Terrific Broth with 5 µl of un-induced culture and your recommended inducer. Cover with a plate lid.**
 3. **Incubate at 37°C and shake at 300 rpm for 12-16 hours.**
 4. **Centrifuge the SeqPrep™ 384 Plate at 2500 rpm for 3 minutes.**
 5. **Remove supernatant by a brief inverted spin.**
 - Immediately, remove cover and decant the supernatant over a tray. Invert the plate onto a paper towel and spin at 600 rpm for 5 seconds.
 6. **Add 30 µl of prepared Lysis Solution/Enzyme Mix.**
 7. **Pipet to mix 8-10 times.**
- OR
- Vortex to mix for 1 minute.**
- Secure the plate to a vortex mixer and gradually increase the vortex speed to obtain a vigorous agitation without splashing liquid from the wells.
8. **Incubate at room temperature for 3 minutes.**
 9. **Remove lysate by pipetting or inverted spin.**
 - For inverted spin, decant the lysate over a tray, invert the plate onto a paper towel, and spin at 2500 rpm for 30 seconds.
 10. **Wash the sample once with 40 µl of prepared Wash Solution.**
 - Add 40 µl of Wash Solution. Mix by shaking or pipetting. Remove solution by pipetting or inverted spin.
 11. **Wash the sample twice with 40 µl of 70% Isopropanol.**
 - Add 40 µl of 70% Isopropanol. Mix by shaking or pipetting. Remove solution by pipetting or inverted spin.
 12. **Invert the plate onto a paper towel or an absorbent pad and centrifuge at 2500 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 SeqPrep™ 384 Plate at room temperature for 30 minutes.**
 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 30 minutes or incubate at 4° C overnight.
 15. **DNA is ready for immediate use.**