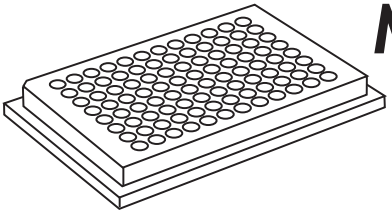

MultiScreen®$_{HTS}$ PLASMID 96-Well Plates
User Guide

For research only
Not for use in clinical applications
Single use only

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MSNU PSD 50 (50/Pk)

Introduction

The MultiScreen$_{HTS}$ PLASMID plates are single-use 96 well filter plates developed for the purification of plasmid and BAC DNA in the 0.035 to 0.3 mL volume range. MultiScreen$_{HTS}$ PLASMID plates are designed for vacuum filtration using the MultiScreen™$_{HTS}$ vacuum manifold.

A novel size exclusion technology allows for the purification of plasmid and BAC DNA without having to bind, wash, and elute. The DNA is concentrated on the surface of the membrane, while the contaminants pass through to waste. After washing, the DNA is recovered from the surface of the membrane.

Bacterial lysates are cleared through the MultiScreen$_{HTS}$ CLEARING plate via filtration and the plasmids in the filtrate are further purified using the MultiScreen$_{HTS}$ PLASMID plate. The plasmids that are retained by the membrane in this plate are washed, resuspended in Solution 5, and withdrawn from the plate.

Materials Required

In order to use the MultiScreen$_{HTS}$ PLASMID plates you will need:

- MultiScreen$_{HTS}$ vacuum manifold (Cat. No. MSVM HTS 00)
- MultiScreen$_{HTS}$ CLEARING plates (Cat. No. MSNA NLY 50)
- Portable vacuum pump (Cat. No. WP62 115 60)

NOTE: Millipore recommends that you use a vacuum pressure pump for vacuum consistency. The pump enables you to maintain constant vacuum to achieve reproducible results.

- Solution 1, cell resuspension solution (Cat. No. LSKC RS5 00)
- Solution 2, cell lysis solution (Cat. No. LSKC LS5 00)
- Solution 3, neutralization solution (Cat. No. LSKN S05 00)
- Solution 4, nuclease-free water for wash (Cat. No. LSKN F05 00)
- Solution 5, tris buffer for storage (Cat. No. LSKC TB5 00)
- RNase A, 30 mg (Cat. No. LSKP MRN 30)

Usage Guidelines

- For research use only. Not for use in clinical applications.
- The MultiScreen$_{HTS}$ PLASMID plate is a disposable single use only device.
- Operate at a temperature of 4 °C to 25 °C.
- Not for use in centrifugal mode.
- For use with vacuum only. Filtration time will vary depending upon the volume added to the wells and the strength of the vacuum source. It is important that all wells are completely emptied of liquid before redissolving purified plasmid DNA.
- Do not vacuum with the lid on the plate.

Preparation

Before beginning the protocol for purifying the DNA, review the following:

- Add RNase (total contents of tube) to Solution 1, mix thoroughly, and store at 2 °C to 8 °C. (All other solutions should be stored at 15 °C to 30 °C.)
- Bring Solution 2 to room temperature before using in order to dissolve detergent that may have precipitated due to lower temperatures during shipping.
- Screw cap on Solution 2 tightly immediately after use in order to avoid destabilization that may occur on exposure to air.

Culturing the Bacteria

The recommended media for culturing bacteria prior to purification of DNA using the MultiScreen$_{HTS}$ PLASMID plates is 2x Luria-Bertani (Miller) broth. This media has twice the content of tryptone and yeast extract compared to normal LB (Miller) broth but has the same salt content. The formulation per liter of media is as follows:

Tryptone	20g
Yeast Extract	10g
NaCl	10g

1. Inoculate *E. coli* host into 1 mL aliquots (for plasmid DNA) or 1.5 mL aliquots (for BAC DNA) of 2X LB plus antibiotic in sterile 96 deep well blocks (2 mL capacity). Cover plates and secure in incubator. Incubate at 37 °C at 320 rpm for 20 hours (for plasmid DNA) or for 20–24 hours (for BAC DNA).
2. Cover the deep well block cultures with clear plate tape (Cat. No. MATA 096 00), and centrifuge at 1500 × g for 5 minutes. After centrifugation, immediately decant culture supernatant to a container for proper disposal. Invert and tap the plates firmly on several layers of paper towels on the bench to remove residual culture supernatant.
3. Resuspend pellets in 150 µL of Solution 1 first using a plate shaker or vortex. Ensure pellets are redissolved.

Plasmid Miniprep Using MultiScreen$_{HTS}$ PLASMID Plates

1. Add 150 µL of Solution 2. Mix immediately and thoroughly with a plate shaker (half speed) for 1 minute. Incubate for an additional 2 minutes at room temperature. Do not exceed 5 minutes.
2. Add 150 µL of Solution 3. Mix immediately and thoroughly (half speed) with a plate shaker for 2 minutes.
3. Place MultiScreen$_{HTS}$ PLASMID plate into vacuum manifold.
4. Pipette 200 µL of the lysate from the bottom of each deep well, and dispense into the corresponding well of a MultiScreen$_{HTS}$ CLEARING plate.
5. Clarify the lysate using vacuum filtration. Adjust the vacuum to 8 inches of Hg (270 millibar/200 torr). Draw the lysate through the MultiScreen$_{HTS}$ CLEARING plate into the MultiScreen$_{HTS}$ PLASMID plate by applying vacuum for 5 minutes or until wells are empty. See manifold user guide for details. Discard MultiScreen$_{HTS}$ CLEARING plate.
6. Place the MultiScreen$_{HTS}$ PLASMID plate on top of the manifold collar. Apply full vacuum (24 inches of Hg) for 5–7 minutes, or until wells are empty. Direct filtrate to waste. When filtration is complete, switch off vacuum.

   NOTE: Filtration time is sample, temperature, and pressure dependent. The filters will appear shiny even after the wells are empty.

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Plasmid Miniprep Using MultiScreen$_{HTS}$ PLASMID Plates, continued

7. Add 200 µL of Milli-Q-grade water or Solution 4 to each well of the MultiScreen$_{HTS}$ PLASMID plate. Apply full vacuum for 3–5 minutes or until wells are empty then turn off vacuum.
8. To resuspend plasmid, add 50 µL of Solution 5 to each well of the MultiScreen$_{HTS}$ PLASMID plate.
9. To resuspend, shake for 5 minutes on a plate shaker. To recover, pipette retained plasmid from the wells of the MultiScreen$_{HTS}$ PLASMID plate (accessing the dissolved plasmid from above) into a V-bottom microplate. To recover samples without shaking, add the resuspension buffer to the wells and let the plate sit for 30 minutes before pipetting.

BAC DNA Purification Using MultiScreen$_{HTS}$ PLASMID Plates

1. Centrifuge deep well blocks at 1500 × g for 10 minutes. After centrifugation, immediately decant culture supernatant to a container for proper disposal. Invert and tap the plates firmly on absorbent paper/pads to remove residual culture supernatant.
   NOTE: Failure to remove media will add undesired volume to lysate.
2. Freeze the plates containing the pellets at -20 °C for 1 hour (if desired the samples can be frozen for up 24 hours prior to processing).
   NOTE: Freezing the pellets improves resuspension and yield of BAC DNA.
3. Allow the pellets to thaw at room temperature for 15 minutes.
4. Resuspend pellets by adding 100 µL of Solution 1 (containing RNase A) to each well then mixing on a plate shaker for 5 minutes until cells are completely resuspended. If the cells are not completely resuspended, increase the shaking time as needed. Alternatively, resuspension may be achieved by vortexing or pipetting.
   NOTE: Thorough resuspension of cells is critical for successful lysis. No pellets should be visible at the bottom of the wells.
5. Add 100 µL of Solution 2 to each well without mixing or shaking. Incubate at room temperature for 5 minutes.
   NOTE: Mixing after the addition of Solution 2 may decrease yield.
6. Add 100 µL of Solution 3 to each well. Mix immediately for 2 minutes using a plate shaker.
7. Pipette the entire volume of lysate up and down three times to break up any large clumps of flocculent.
8. Pipette the entire lysate volume from the bottom of each deep well and dispense into the corresponding well of the MultiScreen$_{HTS}$ CLEARING plate.
9. Clarify the lysate using vacuum filtration. Adjust the vacuum to 8 inches of Hg (270 millibar/200 torr). Draw the lysate through the MultiScreen$_{HTS}$ CLEARING plate into the MultiScreen$_{HTS}$ PLASMID plate by applying vacuum for 5 minutes or until wells are empty. See manifold user guide for details. Discard MultiScreen$_{HTS}$ CLEARING plate.

BAC DNA Purification Using MultiScreen$_{HTS}$ PLASMID Plates, continued

10. Place the PLASMID plate containing clarified lysates on top of the manifold collar. Apply full vacuum (24 inches of Hg) until wells are empty then switch off vacuum.
    NOTE: Filtration time is sample, temperature, and pressure dependent. The filters will appear shiny even after the wells are empty.
11. Add 200 µL of Milli-Q water or Solution 4 to each well of the MultiScreen$_{HTS}$ PLASMID plate. Apply full vacuum until wells are empty then switch off vacuum.
12. Resuspend BAC DNA samples by adding 35 µL of Solution 5 to the wells of the MultiScreen$_{HTS}$ PLASMID plate. After adding Solution 5 to the wells, shake for 10 minutes on a plate shaker.
13. Pipette retained BAC DNA from the wells of the MultiScreen$_{HTS}$ PLASMID plate into the V-bottom plate for storage. The recovery volume can be maximized by tilting the MultiScreen$_{HTS}$ PLASMID plate before collecting the sample.

Storage

Store plate at room temperature.

Specifications

Maximum operating sample capacity: 0.3 mL

Hold-up volume membrane and support: 4 µL

Maximum vacuum pressure: 25 inches Hg/ 635 Torr

Active membrane area of well: 0.2 cm^2

Dimensions of assembled plate:

Plate length:	123.4 mm (4.9 in.)
Plate width:	82.7 mm (3.3 in.)
Plate depth:	14.6 mm (0.6 in.) without cover
	16.5 mm (0.65 in.) with cover

Technical Assistance

In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the U.S., see your Millipore catalogue for the phone number of the office nearest you or go to our web site at www.millipore.com/offices for up-to-date worldwide contact information. You can also visit the tech service page on our web site at www.millipore.com/techservice.

Warranty

This product is covered by Millipore's standard limited warranty, as set forth in Millipore's Bioscience Catalogue.

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