Thomas Scientific

GE Healthcare

illustra AutoScreen-96A Well Plates

for Purification of Sequencing Reactions and other Size Exclusion Applications

Product booklet

Thomas No. CHM01P692 Code 25-9005-98 (10 plates)

Page finder

1. Legal

http://www.gehealthcare.com/lifesciences

GE Healthcare UK Limited.
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The MegaBACE instrument is a confocal scanning system licensed under US Patent Numbers 5,091,652 and 5,274,240, and corresponding foreign patents and patent applications, including any continuations, continuations-in-part, and subdivisions and the like.

The instrument is also an Authorized DNA Sequencer. It is authorized under one or more U.S. Patent Numbers 4,849,513; 5,171,534; 5,015,733; 5,118,800; 5,161,507; 5,118,802; 4,855,255; and 5,36,860, and corresponding foreign patents and patent applications. The purchase of this instrument includes limited, non-exclusive rights under the subject patents to use this instrument for sequencing and fragment length analysis when used with Authorized Reagents. The use of this instrument with Authorized Reagents provides a limited license to perform DNA sequencing and fragment length analysis in accordance with the label rights accompanying such reagents. Purchase of this instrument does not itself convey to the purchaser a complete license to perform DNA sequencing and fragment length analysis under the subject patents. Authorized reagents may be obtained from licensed vendors, or reagents may be authorized under separate license arrangements from PE Applied Biosystems®. The above patent rights are granted solely, for research and other uses that are not unlawful. No other licenses are granted expressly, impliedly, or by estoppel.

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2. Handling and storage

2.1. Safety warnings and precautions

Warning: For research use only.

Not recommended or intended for diagnosis of disease in humans or animals. Do not use internally or externally in humans or animals.

All chemicals should be considered as potentially hazardous. Only persons trained in laboratory techniques and familiar with the principles of good laboratory practice should handle these products. Suitable protective clothing such as laboratory overalls, safety glasses and gloves should be worn. Care should be taken to avoid contact with skin or eyes; if contact should occur, wash immediately with water (see Material Safety Data Sheet(s) and/or Safety Statement(s) for specific recommendations).

2.2. Storage

Store Autoscreen-96A plates horizontally with the package label facing up, and with the foil wrap undisturbed, at 4°C. Under these conditions, the performance of the product will not be compromised for up to eight months from the date of manufacture. The plates may also be stored at ambient temperature for up to 2 months from date of receipt. Do not store in a freezer.

2.3. Expiry

For expiry date please refer to outer packaging label.

3. Components

3.1. Kit contents

The following components are included in the product:
Autoscreen-96A plates containing DNA Grade Sephadex™ G-50 rehydrated in double-distilled water with 0.05% Kathon™ CG/ICP Biocide added as a preservative..

3.2. Materials and equipment to be supplied by user

- Beckman Allegra™ 6 Series centrifuge or equivalent.
- Beckman GH-3.8A horizontal rotor or equivalent.
- Beckman MicroPlus™ Multiwell Plate Carriers (catalog #361304) or equivalent.
- Adaptor plate (catalog# MSABI9602) from millipore corporation.

4. Description

4.1. Introduction

AutoScreen-96A consists of a 96-well filter plate containing DNA Grade Sephadex G-50 for purification of sequencing reactions prior to analysis on MegaBACE™ 1000 or ABI™ Prism™ automated sequencing instruments and can be used for other size exclusion applications.

Note: Efficient removal of unincorporated dye terminators labelled with Big Dye™ v.3 requires a single additional wash step as described below. If this wash step is not performed, a large accumulation of dye will elute with the sequencing reaction products and obscure data generated on ABI DNA sequencers. When purifying reactions containing Big Dye v.3, do not perform step 5.1.6. Instead, perform steps 5.1.6(a)–5.1.6(f).

5. Protocols

5.1. Preparation of AutoScreen-96A plates

5.1.1 Remove the AutoScreen-96A plate from the foil storage pouch.

Note: If the plate was stored at 4°C, allow it to equilibrate to ambient temperature in the foil pouch before use (~ 2 h).

5.1.2 Remove both the top and bottom adhesive seals.

Note: Once the bottom seal is removed, keep the AutoScreen-96A plate on top of a collection plate. Do not allow the bottom surface of the AutoScreen-96A plate to come in contact with laboratory benchtop liners, wipes, or other materials.

5.1.3 Assemble the collection plate, adapter plate for AutoScreen-96A (if necessary), and AutoScreen-96A plate. Place the assembled unit into a centrifuge which can accommodate swinging 96-well plate carriers.

5.1.4 Centrifuge the assembled unit for 5 min at 910 × g (2240 rpm for a Beckman Allegra 6 Series centrifuge using a GH-3.8A horizontal rotor).

5.1.5 Add dropwise 150 µl of distilled water and centrifuge again as in 5.1.4.

5.1.6 Remove the collection plate containing the hydration medium and replace it with a new U- or V-bottom collection plate. When purifying reactions containing Big Dye v.3, do not perform step 5.1.6. Instead, proceed to step 5.1.6(a).

Note: Be careful not to disturb the Sephadex G-50 column that was created during centrifugation.

Note: The plates used to collect the hydration medium may be reused repeatedly for this same purpose. Check each plate before reusing to ensure that no fluid remains from previous centrifugations.

Modifications to purify reactions containing Big Dye v.3

5.1.6(a) Remove the collection plate containing the hydration medium. Discard this eluate appropriately.

5.1.6(b) Place this same collection plate under the AutoScreen-96A plate. Do not use a new collection plate.

5.1.6(c) Pipette 150 µl of 1 mM EDTA, pH 8.0 into each well of the AutoScreen-96A.

5.1.6(d) Centrifuge the assembled AutoScreen-96A at 910 × g for 5 min.

5.1.6(e) Remove the collection plate containing the eluate from the above 5 min spin. Discard the eluate and save the collection plate for washing and reuse in other purifications.

5.1.6(f) Place a new or cleaned collection plate under the AutoScreen-96A. Continue as per step 5.2.

5.2. Purification of sequencing samples for loading onto MegaBACE 1000 or ABI Prism sequencing instruments

5.2.1 Bring the volume of each sequencing reaction to 15 µl using distilled water, if necessary.

Note: The preferred loading volume is 15–20 µl. The suggested range for loading volumes to achieve optimum purification is 10–20 µl.

5.2.2 Slowly apply each sequencing reaction to the center of the column resin bed in the wells of the AutoScreen-96A plate.

Note: It is critical that the samples be applied to the centers of the G-50 columns in a drop-wise fashion.

5.2.3 Centrifuge the samples for 5 min at 910 × g.

Note: A plate lid may be used during this centrifugation step to improve consistency of volume recovery.

5.2.4 Proceed with the standard sample loading procedures for MegaBACE 1000 or ABI Prism sequencing instruments.

Note: See Appendix 1 for suggested MegaBACE loading conditions.

6. Appendices

6.1. Suggested loading conditions for MegaBACE 1000

Injection parameters
10–20 µl sample volume: 3 kV for 75 s
Electrophoresis conditions
Medium read-length of 500–700 bases: 8 kV for 120 min
Long read-length of > 700 bases: 5 kV for 300 min

Note: To inject sequencing reactions from MegaBACE loading buffer, it is necessary to first dry the samples and then resuspend each well in 10 µl of buffer. Injection parameters and electrophoresis conditions need not be varied from those suggested above.

Note: It is normal for signal intensities from AutoScreen-96A-purified sequencing reactions to be two to three times lower than signal intensities from ethanol-precipitated reactions; however, read-lengths will not be affected.

6.2. Suggested loading conditions for ABI Prism 377

Dry the samples in the collection plate for 10–15 min using low heat in a speed vac.
Resuspend each sample in 4 µl of formamide loading dye.
Load 1.5–2.0 µl in each well of a gel.

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25-9005-98PL Rev B 10/2008