OROCHEM
Molecular Purification by Design

INSTRUCTIONS

Resin Seeker 96-Well Plates Kits

Product Description: Resin Seeker 96-well plates are available for the following chromatography modes:

Mode	Description	Product Number	Package Description
Anion Exchange Chromatography	AIEX 96-well plate kits	OC41MDAEX-96	Mixed anion exchange plate (20 µL resin beds)
Cation Exchange Chromatography	CIEX 96-well plate kits	OC41MDCEX-96	Mixed cation exchange plate (20 µL resin beds)
Hydrophobic Interaction Chromatography	HIC 96-well plate kits	OC41MDHIC-96	Mixed hydrophobic interaction plate (20 µL resin beds)
Mixed Mode Chromatography	MMC 96-well plate kits	OC41MDTRP-96	TOYOPEARL MX-Trp-650M plate (20 µL resin beds)

The resin is supplied in 20% ethanol. Each kit includes both a wash and a collection plate.

Storage: Upon receipt, store at 4 ºC. Product shipped at ambient temperature.

Introduction

Resin Seeker 96-well plates are disposable filter plates packed with TOYOPEARL® and/or TOYOPEARL GigaCap® resins from Tosoh Bioscience and are available in several configurations for ion exchange, HIC, and mixed-mode chromatography. Resin Seeker 96-well plates can be used to screen multiple steps of the purification process including binding, wash, and elution conditions in addition to resin selectivity, binding kinetics, purity, and recovery of your target molecule. Resin Seeker plates can be operated manually using a multi-channel pipette or in an automated system designed for high throughput screening in a 96-well plate format.

All TOYOPEARL and/or TOYOPEARL GigaCap resins used in the Resin Seeker 96-well plates are also available in ToyoScreen® pre-packed columns and as bulk media. This allows seamless scale-up and process optimization once resin screening is complete.

Important Product Information

- For resin description, plate configuration and additional information (data), please see the attached product flyer.
- If the purification is performed using centrifugation, the spin plate must be balanced throughout the procedure with a duplicate plate or a balance plate filled with an appropriate volume of water.
- Empirically determine the optimal buffer (pH and salt concentration) for purifying and eluting the protein of interest based on the pI and hydrophobicity of the protein.
- When using Resin Seeker 96-well plates with an automated system, follow instructions provided by the equipment manufacturer with regards to plate set-up and method programming.

Additional Materials Required

- Variable-speed centrifuge with rotor and carriers capable of handling stacked plates (4.4 cm height) at 500 × *g* or a vacuum manifold system. Suggested flow rates are 4-8 inches Hg (2-4 psi), which is equivalent to 1-2 drops/second.
- Multi-channel pipettor and tips

Procedure for Manual Operation of Resin Seeker 96-well Plates Kits

1. Equilibrate Resin Seeker Plate to room temperature.
2. Use the tab to remove the sealing material from the bottom of the plate. Place the Resin Seeker Plate on top of a wash plate.
3. Remove the sealing material from the top of the Resin Seeker Plate.
4. Place the assembly into a centrifuge with a 96-well plate-carrier rotor and centrifuge at 500 × *g* for 2 minutes to remove the storage buffer. Discard the flow-through.
5. Add appropriate equilibration buffer to each well and allow time for the resin to settle before removing buffer by centrifugation. For examples of equilibration buffer, please refer to the following table:

Plate Type	Equilibration/Wash Buffer
AIEX 96-Well Plate	Tris-HCl, pH 8.7
CIEX 96-Well Plate	0.05 mol/L acetate buffer, pH 4.7
HIC 96-Well Plate	0.1 mol/L phosphate buffer, 1.8 mol/L sodium sulfate, pH 7.0
MMC 96-Well Plate	0.05 mol/L acetate buffer, 0.1 mol/L NaCl, pH 4.7

6. Repeat STEP 5 at least three times or until the resin is fully equilibrated.
7. Place the purification plate on top of the wash/collection plate.
8. Load 20 to 100 µL of clarified sample into each well. Larger sample volumes can be loaded in additional aliquots.
9. Incubate the plate. Incubation time for each sample will be application/molecule specific and needs to be optimized. For small sample volumes (*e.g.* 20 µL), apply 20 µL of loading buffer on top of the resin bed after the sample has fully absorbed to ensure maximum protein recovery.
10. After appropriate incubation, centrifuge at 500 × *g* for 2 minutes.
11. Wash out the unbound sample at least three times with 200 µL of equilibration/wash buffer per well and centrifuge at 500 × *g* for 2 minutes. Remember to empty the wash plate between each step.
12. Place the purification plate on a new collection plate. Add 200 µL of elution buffer to each well and centrifuge at 500 × *g* for 2 minutes.
13. Though the number of elution steps needed to fully elute your target molecule will vary, a total of three, 200 µL additions should be sufficient in most cases. For examples of elution buffer, please refer to the following table:

Plate Type	Elution Buffer
AIEX 96-Well Plate	Tris-HCl, pH 8.7 + NaCl (up to 1 mol/L)
CIEX 96-Well Plate	acetate buffer, pH 4.7 + NaCl (up to 1 mol/L)
HIC 96-Well Plate	0.1 mol/L phosphate buffer, pH 7.0
MMC 96-Well Plate	Tris-HCl, 0.3 mol/L NaCl, pH 8.5