

INSTRUCTIONS

Pierce[®] 96-well Microdialysis Plate

88260 88262

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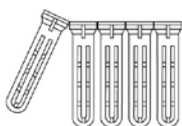
Number	Description
88260	Pierce 96-well Microdialysis Plate, 10K MWCO , 12 cartridges of eight microdialysis devices in a 96 deep-well plate
88262	Pierce 96-well Microdialysis Plate, 3.5K MWCO , 12 cartridges of eight microdialysis devices in a 96 deep-well plate

Storage: Upon receipt store at room temperature (RT). Product shipped at ambient temperature.

Introduction

The Thermo Scientific Pierce 96-well Microdialysis Plate is a convenient, disposable and rapid dialysis device for simultaneous processing of one to 96 samples of 25-100 μ L each. Each microdialysis device has two regenerated cellulose 10K or 3.5K molecular weight cut-off (MWCO) membranes separated by < 2mm. The low-binding regenerated cellulose membranes are rated to retain proteins and other macromolecules that are larger than the MWCO, while allowing removal of buffer salts and small contaminants. The combination of short diffusion distance and large surface area allows for rapid dialysis with the removal of salts and small molecules often completed in 2-4 hours. In addition, due to surface tension, the small distance between the membranes allows easy and highly efficient sample recovery using only standard laboratory pipettes. The dialysis chambers are provided as strips of eight attached units that can be easily separated. With this feature, waste is eliminated by only using the needed number of chambers. Dialysis can be efficiently performed in a standard 96 deep-well plate that uses a minimal amount of buffer while still providing > 95% removal of small molecules (see Protocol Summary and Additional Information sections). The assembled device is ideal for high-throughput applications as it is compatible with standard 96-well laboratory equipment and automated liquid-handling systems.

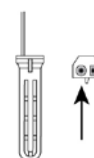
Procedure Summary



1. Remove one or more devices, as needed. If only one device is required, break it carefully from the 8-segmented cartridge.



2. Add dialysis buffer to a deep-well plate ($\leq 1800\mu$ L) or a 2mL microcentrifuge tube ($\leq 1400\mu$ L) and set aside.



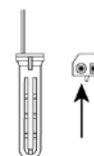
3. To load each device, insert an upright pipette tip filled with sample into the round opening (see arrow). Slowly add the sample (10-100 μ L).



4. Place device into the deep-well plate or 2mL microcentrifuge tube containing buffer.



5. Dialyze to remove low molecular weight compounds (1 hour to overnight). Change dialysis buffer as required. Shake plate gently on a plate shaker (optional).



6. Remove device from plate or tube and recover sample. Set pipette volume to 140 μ L, insert upright pipette tip into round opening of device and slowly withdraw the sample.

Important Product Information

- To prevent contamination, do not touch the membrane with ungloved hands.
- Once wet, do not let the membrane become dry.
- The microdialysis devices can be used individually in 2mL microcentrifuge tubes. (optional)
- If the sample density is $\geq 1.150\text{g/mL}$, such as protein in saturated 4.1M $(\text{NH}_4)_2\text{SO}_4$, 45% sucrose or 8M GuHCl, use $\leq 50\%$ of the maximum sample volume, which allows for the influx of water during dialysis and ensures the device does not over fill. Performing serial dialysis using buffers with decreasing concentrations of solutes (salt) will prevent the osmotic pressure from overfilling the device (e.g., dialyze a 5M NaCl sample against a buffer with 0.5M NaCl).
- For high-throughput drug-binding experiments using equilibrium dialysis, we recommend using the Thermo Scientific RED Device Single-Use Plate with Inserts (Product No. 90006).

Additional Materials Required

- 96-well deep-well plate, 2.2mL (Product No. 88261)
- Pipette for sample recovery
- Plate shaker (optional)
- 2mL microcentrifuge tubes (optional)

Procedure for using the Pierce 96-well Microdialysis Plate

1. Remove the required number of microdialysis devices from the plate. Individual devices can be detached from the eight-segment cartridges. To prevent membrane contamination, handle the device at the top or sides using gloves.
2. Add $\leq 1800\mu\text{L}$ of dialysis buffer to the appropriate number of wells in a 96 deep-well plate or $\leq 1400\mu\text{L}$ of dialysis buffer to a 2mL microcentrifuge tube and set aside.
3. Add $100\mu\text{L}$ of dialysis buffer to each microdialysis device by slowly adding the buffer through the round opening of the device.
4. Remove the buffer from the device by setting the pipette volume to $140\mu\text{L}$, inserting the pipette tip into the round opening and slowly aspirating the buffer. Do not let the membrane become dry.
5. Load the sample ($10\text{-}100\mu\text{L}$) slowly as described in Step 3. Confirm that the sample is settled at the bottom of the device, especially when loading a small volume (e.g., $10\mu\text{L}$), by carefully pushing the sample down with air through the pipette.
6. Place the device into the deep-well plate or 2mL microcentrifuge tube containing the buffer (Step 2). Cover the top (sample loading portion) of the device with laboratory film.
7. Shake the plate gently on a plate shaker (optional).
8. Dialyze for an amount of time sufficient to remove low-molecular weight compounds for the specific downstream application (1 hour to overnight). A typical dialysis using two buffer changes takes less than 5 hours to remove salts (e.g., 1M NaCl, Figure 1); however, dialysis time will vary depending on the salt and small molecule concentrations. A typical dialysis procedure is as follows: dialyze for 2 hours at room temperature or 4°C ; change the dialysis buffer and dialyze for another 2 hours; if needed, change the dialysis buffer and dialyze overnight. To change the buffer, move the microdialysis device into a new deep-well plate channel or use a new microcentrifuge tube.
9. Remove the device from the plate and recover the sample as described in Step 4.

Troubleshooting

Problem	Possible Cause	Solution
Small molecule was not completely removed	Buffer was not changed	Dialyze for two hours at RT or 4°C; change the dialysis buffer and dialyze for another two hours; change the dialysis buffer and dialyze overnight
Sample volume was significantly increased	Sample density was $\geq 1.150\text{g/mL}$, such as protein in saturated $4.1\text{M } (\text{NH}_4)_2\text{SO}_4$, 45% sucrose or 8M GuHCl	Use $\leq 50\%$ of the maximum sample volume

Additional Information

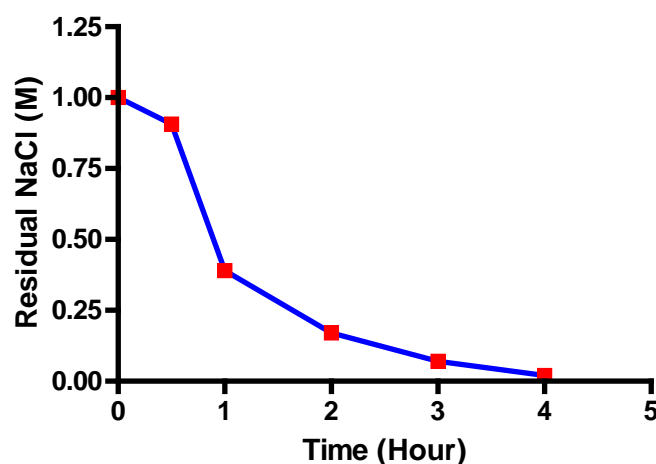


Figure 1. Rate of removal of NaCl in the Thermo Scientific Pierce 96-well Microdialysis Device. Samples of 0.1mL (0.4mg/mL cytochrome c containing 1M NaCl) were dialyzed against 1.8mL of water in a 96 deep-well plate at RT with gentle shaking on a plate shaker. The buffer was changed at 1-, 2- and 3-hour intervals over a 4-hour period. The rate of NaCl removal was determined by measuring the conductivity of the retentate at the indicated time intervals. Removal of NaCl was > 83% after 2 hours and > 99% after 4 hours.

Thermo Scientific Pierce 96-well Microdialysis Membrane Chemical Compatibility List

Acetic acid, 96%	G	Formic acid, 25%	G	Phosphoric acid, 25%	L
Acetonitrile	G	Formic acid, 100%	N	Phosphoric acid, 85%	N
Acetone	G	Glycerol	G	Isopropanol	G
Ammonium hydroxide (1N)	L	Hydrochloric acid, 10%	L	1-Propanol	G
Ammonium hydroxide, 25%	L	Hydrochloric acid, 25%	N	Potassium hydroxide (1N)	L
Chloroform	G	Hydrofluoric acid, 50%	N	Potassium hydroxide, 32%	N
Dimethyl sulfoxide	G	Methanol, 98%	G	Sodium hydroxide (1N)	L
Ethanol, 98%	G	Methylene chloride	G	Sodium hydroxide, 32%	N
Ethylacetate	G	Nitric acid, 25%	N	Sulfuric acid, 98%	N
Ethylene glycol	G			Tetrahydrofuran	G
				Toluene	G

Legend

G = Good chemical resistance

L = Limited chemical resistance (pore size not guaranteed)

N = No chemical resistance, not recommended

Related Thermo Scientific Products

88261	96-well Deep-Well Plate, 2.2mL, 1/pkg
90006	RED Device Single-Use Plate with Inserts
69580	Slide-A-Lyzer® MINI Dialysis Unit, 2K MWCO, 50/pkg
69550	Slide-A-Lyzer MINI Dialysis Unit, 3.5K MWCO, 50/pkg
69560	Slide-A-Lyzer MINI Dialysis Unit, 7K MWCO, 50/pkg
69570	Slide-A-Lyzer MINI Dialysis Unit, 10K MWCO, 50/pkg
69590	Slide-A-Lyzer MINI Dialysis Unit, 20K MWCO, 50/pkg
28372	BupH™ Phosphate Buffered Saline Packs, 40 packs
28376	BupH Tris Buffered Saline Packs, 40 packs
88269	Sealing Sheet for 96-well Microdialysis Plate, 1/pkg

This product ("Product") is warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation") and to be free from defects in material and workmanship. Unless otherwise expressly authorized in writing, Products are supplied for research use only. No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than the original purchaser of the Product ("Buyer").

No other warranties, express or implied, are granted, including without limitation, implied warranties of merchantability, fitness for any particular purpose, or non infringement. Buyer's exclusive remedy for non-conforming Products during the warranty period is limited to replacement of or refund for the non-conforming Product(s).

There is no obligation to replace Products as the result of (i) accident, disaster or event of force majeure, (ii) misuse, fault or negligence of or by Buyer, (iii) use of the Products in a manner for which they were not designed, or (iv) improper storage and handling of the Products.

Current product instructions are available at www.thermoscientific.com/pierce. For a faxed copy, call 800-874-3723 or contact your local distributor.

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Москва

 ул. Магаданская, д. 7, к. 3 ■ тел./факс: (495) 745-0508 ■ sales@dia-m.ru
www.dia-m.ru

С.-Петербург
 +7 (812) 372-6040
spb@dia-m.ru

Новосибирск
 +7 (383) 328-0048
nsk@dia-m.ru

Воронеж
 +7 (473) 232-4412
vrn@dia-m.ru

Йошкар-Ола
 +7 (927) 880-3676
nba@dia-m.ru

Красноярск
 +7 (923) 303-0152
krsk@dia-m.ru

Казань
 +7 (843) 210-2080
kazan@dia-m.ru

Ростов-на-Дону
 +7 (863) 303-5500
rnd@dia-m.ru

Екатеринбург
 +7 (912) 658-7606
ekb@dia-m.ru

Кемерово
 +7 (923) 158-6753
kemerovo@dia-m.ru

Армения
 +7 (094) 01-0173
armenia@dia-m.ru

