Ordering FAQs

1. What are my options for placing an order?
2. What payment options are available?
3. Do you have agents in my country?
4. How soon can you ship my order?
5. Will you ship “Collect”?
6. Can I specify the shipping mode?
7. What is the typical delivery time for international orders?

Operating FAQs

1. What is the difference between the HTD 96a and HTD 96b?
2. How should I clean the Teflon block?
3. Can the Teflon block be autoclaved?
4. Can the Teflon block be ultrasonically cleaned?
5. What incubation temperatures and shakers can I use?
6. How can I mark Teflon blocks uniquely and enhance the legibility of the letters?
7. How is the Membrane Dialysis Strips Molecular Weight Cut Off (MWCO) pore size determined?
8. How long can dry membranes be stored and what is proper membrane hydration and preparation?
9. How long can we store membranes in 20% ethanol after hydration?
10. What volumes can be used in the HTD 96a/b unit?
11. What causes “leakage” of proteins across the dialysis membrane?
12. What causes physical “leakage” after extended use?
13. Is there a specific expiry date for the Teflon blocks?
14. What is the time required to reach equilibrium?
15. What are the dimensions and utility requirements for the HTD 96a/b?
16. Can the HTD 96a/b be used on robotic workstations?
17. What causes difficulty in closing the clamp?
18. Can I use radio isotopes in the HTD 96 unit?
19. Can you provide a generic protein binding protocol?
20. What membrane pore sizes are most commonly used?

Ordering FAQs

1. **What are my options for placing an order?**
   - Fax a purchase order to HTDialysis at (01) 860-464-2029
   - E-mail a PDF purchase order to info@HTDialysis.com
   - Order through International Scientific suppliers including VWR International and Fisher Scientific

2. **What payment options are available?**

   Bank check, wire transfer or Electronic Fund Transfer (ETF).

3. **Do you have agents in my country?**

   We do not have any exclusive HTDialysis agents, however, we have non-exclusive agents in the following countries:

India

Sanchayita Kar
Viswagen Biotech Pvt. Ltd.
23/863G, Thazhathuveettil Buildings,
Market Road, Pala- 686575, Kerala, India
E-mail: sanchayita.kar@viswagen.com

Japan

Double Helix International, Inc.
1046 Princeton Drive, Unit 202
Marina del Rey, CA 90292
E-mail: hyamamoto@d-helixintl.com

or

Wakamori Shokai Co., LTD.
6-12 Yushima 4-chome,
Bunkyo-ku, Tokyo 113-0034
Japan
E-mail: tsekikawa@wkm.co.jp

or

Funakoshi Co., Ltd.
9-7 Hongo 2-Chome Bunkyou-Ku, Tokyo 113-0033, Japan
Technical support
Phone: +81-3-5684-1620
Fax: +81-3-5684-1775
e-mail: reagent@funakoshi.co.jp
URL: http://www.funakoshi.co.jp

Italy

EOS S.r.l.
Via Fossona 7/A, Cervarese Santa Croce
35030 - Padova - ITALIA

Phone: +39 049 9915554 interno 244
Fax +39 049 9919469
e-mail: mariette.buy@eosbio.com

4. **How soon can you ship my order?**

   Orders are typically shipped within 24-48 hours of receipt and are accompanied by an e-mail providing the purchasing agent the carrier tracking number.

5. **Do you ship "Collect"?**

   Yes, we ship "Collect" please provide an account number with one of the following carriers DHL, Fed Ex, or UPS.

6. **Can I specify the shipping mode?**

   Yes, we ship all USA orders by; overnight, 2-day, or ground while all International orders are shipped Express, Priority or Worldwide Expedited.

7. **What is the typical delivery time for international orders?**

   Orders are generally delivered within 48-72 hours barring any Customs delays.

Operating FAQs

1. **What is the difference between the HTD 96a and HTD 96b?**

   - HTD96a original design had sufficient pressure to seal if the unit was only closed during operation and stored in the open, non-compressed state. The design was changed in response to leakage detected by two customers who left the unit closed for prolonged periods resulting in the Teflon remaining "compressed" and sealing inefficiently.

   - HTD96b was introduced to eliminate potential "user error" by increasing the thickness of the pressure plate so that even if the unit was inadvertently left closed it still has sufficient pressure to seal completely and reliably.

2. **How should I clean the Teflon block?**
   After each use the HTD dialysis block should be cleaned thoroughly with a non-ionic detergent. Disassemble the dialysis block and soak overnight in a 2-liter beaker containing a 2% solution of Micro 90 (VWR international, Catalog # 21830-416). Rinse the Teflon bars in the 2 liter beaker followed by holding each Teflon bar under a running distilled water stream. Care must be taken to ensure that all detergent has been rinsed from the unit as residual detergent could compromise future binding studies. The Teflon block may also be disinfected with a 10% v/v solution made using commercial Clorox bleach which is 5.25% sodium hypochlorite. The final solution would this be 0.525% sodium hypochlorite solution. Never use any abrasive or brush for cleaning the Teflon blocks as they will cause micro-striations and prevent effective sealing.

3. **Can the Teflon block be autoclaved?**
   Although the current data is limited it indicates that 2 autoclave cycles cause only some discoloration with no adverse effects on performance.

4. **Can the Teflon block be ultrasonically cleaned?**

   One scientist has successfully used the Steris Amsco® Sonic Energy Console, Model #SC1224GD with a ~1% detergent solution (TergajetTM Low-Foaming Phosphate-Free Powdered Detergent) added manually to the cleaning chamber with a 7 minute cycle preprogrammed and the plumbed detergent setting OFF to semi-automate the washing of the Teflon blocks. After the wash cycle the wash water is drained from the wash chamber and the chamber refilled with fresh/clean hot water. The Teflon bars in the wash basket are manually immersed and removed several times for the initial rinsing. This manual rinsing in the wash chamber is critical as any residual detergent can adversely affects binding results. The final rinse uses a 30 min preprogrammed cycle using the rinse/dry cycle in the rinse chamber. This process has been successfully used for more than 30 cycles without any deleterious affects on the Teflon blocks.

5. **What incubation temperatures and shakers can I use?**
   The dialysis block can be incubated at any desired temperature between 20ºC and 45ºC. Equilibrium is reached more rapidly if the dialysis block is shaken during the incubation period. Shaking at 80-100rpm is sufficient using any general incubator containing an orbital or reciprocating platform shaker e.g. Fisher Scientific (Catalog # 14-278-104) and VWR international (Catalog # 47742-750 or #33998-360).

6. **How can I mark Teflon blocks uniquely and enhance the legibility of the letters?**
   Use a colored marker pen and color the blocks over and around the letters. The color generally does not penetrate the letters but does color the surface. After allowing time to dry, thoroughly wipe the surface thereby removing some of the color. This usually results in a faint coloration that contrasts with the white of the letters making them more legible. This color will remain through several washes and should facilitate reading the letters. Another advantage is that if you use different colors for each block you have also quickly identified the correct bars for each set if they get mixed during washing.

7. **How is the Membrane Dialysis Strips Molecular Weight Cut Off (MWCO) pore size determined?**
   Dialysis membranes consist of a matrix of cross-linked polymers. The pore rating, Molecular Weight Cut Off (MWCO), is an indirect measure of the retention performance using a series of standard molecules with varying molecular weights after 17 hours of dialysis. The membrane MWCO is determined as the solute size that is retained by at least 90%. However, since a solute's permeability is also dependent upon molecular shape, degree of hydration, ionic charge and polarity, we recommend selecting a MWCO that is at least half the size of the MW of the species to be retained and/or twice the size of the MW of the species intended to pass through.

8. **How long can dry membranes be stored and what is proper membrane hydration and preparation?**
   The dry membranes can be stored for up to 2 years in sealed or Ziploc bags at 4ºC or ambient temperature. Storage in such bags prevents membranes from drying out and losing their integrity.

   Membrane hydration and preparation:
   - Use only sterile buffers to prepare your membranes before use. This will ensure that microbial contamination will not compromise membrane integrity.
   - Never store hydrated membranes in any buffer without an effective anti-microbial agent e.g. 0.1% sodium azide, 1%sodium benzoate or 1%formaldehyde.

- Never let hydrated membranes dry as that irreversibly changes the pore structure and results in loss of membrane integrity.

9. **How long can we store membranes in 20% ethanol after hydration?**
   We recommend storing them no longer than 4 weeks in 20% ethanol at 4ºC if the initial buffer was sterile. The ethanol is initially added because it helps to remove any glycerin which is added to the membrane during manufacturing to help promote hydration. The key point is that one must avoid any bacterial growth as they may produce cellulases that modify/destroy the membranes.

10. **What volumes can be used in the HTD 96a/b?**
    Volumes from 25µl to 150µl can be used in each side of the dialysis well with a maximum total volume of up to 300ul. Detection sensitivity will often dictate the appropriate volume required.

11. **What causes "leakage" of proteins across the dialysis membrane?**
    Loss of membrane integrity during an experiment will manifest as the presence of proteins in the dialysate and a violation of protein mass balance for the well. This may be caused by microbial contamination and enzymatic degradation of the cellulose membrane. The remedy is to ensure correct membrane preparation and use – see above (**Membrane hydration and preparation**) and this includes thorough cleaning of the Teflon blocks between uses – (**How should I clean the Teflon block**). If "leakage" persists sterilize the Teflon block by autoclaving and repeat the experiment to confirm that the Teflon block was contaminated and caused the leakage.

12. **What causes physical "leakage" after extended use?**
    The sealing of the unit depends on the compression of the Teflon blocks by the pressure plate. The most common cause of leakage after extensive use is micro-striations on the Teflon bars due to inappropriate washing with abrasives or brushes. Replacement of the Teflon block remedies this cause. Another potential cause is rusting of the disc springs in the stainless steel pressure plate. Although all components are stainless steel the grades are different and exposure of the pressure plate to aqueous, acidic conditions may cause parts to rust over time. The remedy is to replace the disc springs, Cat # 1008-01. If the Teflon block in HTD 96a units is left under clamp pressure when not in use it does not "relax" back to its original size thereby resulting in "leakage" when next used. This constraint does not apply to HTD96b units.

13. **Is there a specific expiry date for the Teflon blocks?**
    There is no specific expiration period for the Teflon blocks as it will be dependent on overall use and handling.

14. **What is the time required to reach equilibrium?**
    This is dependent on several factors, incubation temperature, compound structure, and shaking. Most compounds reach equilibrium in less than 6 hours at 37ºC shaking at 80 rpm. We recommend a simple kinetic experiment with compound spiked into buffer and dialyzed against buffer to evaluate the equilibrium time required prior to initiating any binding experiments.

15. **What are the dimensions and utility requirements for the HTD96b?**
    The device is a small bench top, manually operated unit with the following dimensions, 6.7" x 4.6"x 1.5" and weight is 1.2lbs. It does not require any utilities or peripherals and it does not require maintenance or service. The 96-well Teflon block conforms to the SBS standards for 96 well plate well centers.

16. **Can the HTD 96 be used on robotic workstations?**
    The HTD96b has a standard SBS 96-well base that is compatible with most robotic workstations. Many users have successfully automated their assays using a variety of commercial workstations, including those from the following manufacturers ApricotDesigns, Tecan, Hamilton, Packard, and Beckman.

17. **What causes difficulty in closing the clamp?**
Inadvertently using double membranes in the Teflon block instead of ensuring they are separated after hydration.

18. **Can I use radio isotopes in the HTD 96 unit?**
Yes radio isotopes are used and standard decontamination procedures using "COUNT-OFF" from Perkin Elmer followed by standard cleaning protocols are recommended. However, care must be taken with the stainless steel pressure plate to avoid rusting the disc springs. If feasible do not wash or soak the pressure plate. Consider the option of lining the device cavity with a thin plastic (Saran wrap or equivalent food sealing plastic wrap). This will protect the entire device and all you need decontaminate is the Teflon block.

19. **Can you provide a generic protein binding protocol?**
After assembling dialysis apparatus following the Operating Instructions:
1. Add 150ul of phosphate buffer or protein free serum to the receiving side of the dialysis well.
2. Add 150ul of serum (pH adjusted to 7.4uM) spiked with 10uM test compound to the Sample side of the dialysis well.
3. Dialyze for 6 hours at 37ºC with shaking at 80 rpm
3. Acetonitrile (ACN) precipitate and dilute samples prior to analysis in 1.2ml polypropylene tubes.
    A) Remove 10uL from the sample side of the dialysis well and add to 1.2ml tube containing 90uL of phosphate buffer + 300ul of ACN.
    B) Remove 90uL from the buffer side of the dialysis well and add to 1.2ml tube containing 10uL of clean serum + 300uL of ACN.

The samples are then quantitated via the Mass Spec / HPLC. When samples are diluted and extracted in this manner, all samples are in a common matrix and the peak height/area can be directly compared.
All values from the Mass Spec / HPLC are corrected for sample dilution (dilution by ACN is ignored because it is the same for all samples). To correct for dilutions, values from the sample side are multiplied by 10 and values from the buffer side are multiplied by 1.1.
A standard curve can be generated to demonstrate that there is a correlation between peak height/area and compound concentration if desired.
Calculated values:
Fraction unbound = fu = concentration on the buffer side / Concentration on the sample side. These can be peak height or area values corrected by the dilution factor as outlined above.

20. **What membrane pore sizes are most commonly used for serum protein binding studies?**

- 12-14K MWCO ~ 81%
- 6-8K MWCO ~13%
- 10K WWCO ~3.1%
- 3.5K MWCO ~2.4%

Other membranes have been used for special applications:

- 25K MWCO ~0.4%
- 50K MWCO ~0.13%