Reusable 96-well Micro-Equilibrium Dialysis Device 'HTD 96'
A unique patented high throughput equilibrium dialysis apparatus
for cost effective assay up to 96 samples simultaneously

The HTD 96 design combines ease-of-use, versatility, reliability, and precision to expand your options in all micro-equilibrium experiments.

FEATUERES
- **Ready access to both assay and dialysate** from the open top of the wells. The novel vertical alignment of the dialysis membranes eliminates problems associated with trapped air pockets and permits ready access to both the sample and dialysate sides of the membrane from the top of the apparatus. This feature also ensures easy semi-automation as well as full automation using standard robotic workstations or 96-well pipettors.
- **Teflon construction** ensures minimal non-specific binding to the apparatus. Volume chamber suits 25-150µL samples

BENEFITS
- Can be assembled in minutes
- Easily cleaned
- Reusable
- Wide selection of dialysis membranes
- Compatible with standard 96-well laboratory equipment and supplies
- 30-150 ul working volumes
- Automation friendly design

www.apricotdesigns.com
Dialysis system

HTD 96b - Complete Unit (25-150µL samples)  
(Excludes Dialysis Membrane Strips)  
Catalog # 1006  Price $3,468 -201410

HTD 96c - Complete Unit (Faster kinetic, 25-75µL samples)  
(Excludes Dialysis Membrane Strips)  
Catalog # 1009

HTD96a, the original design, needed to be stored in the open, non-compressed state. The design was changed. HTD96b is our standard model. Although still recommended to be stored in the open state, it has sufficient pressure to seal completely and reliably even if the unit is inadvertently left closed for extended periods.

The HTD96c version is designed with reduced well volume design to minimize assay sample volumes (25-75ul) and reagent costs, and reduce the time to reach equilibrium (decreased diffusion path length and increased dialysis membrane surface area to sample volume ratio). The smaller well requires greater precision when pipetting.

Both the HTD96b and HTD96c Teflon blocks use the same base clamp so if you purchase one model and decide to switch, you only need to purchase the alternative Teflon block rather than another complete unit.

**Accessory parts**

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<th>HTD 96b - Base</th>
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<tr>
<th>HTD 96b - Stainless Steel Pressure Plate</th>
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<tr>
<th>HTD 96a/b - Stainless Steel Connecting Rods</th>
<th>HTD 96a/b - Stainless Steel Disc Springs</th>
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### Membrane consumables:

<table>
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<tr>
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<th>Name</th>
<th>MWCO</th>
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<tr>
<td>1135</td>
<td>Regenerated Cellulose - MWCO 3.5K</td>
<td>50u/pack</td>
</tr>
<tr>
<td>1103</td>
<td>Regenerated Cellulose - MWCO 6-8K</td>
<td>50u/pack</td>
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<tr>
<td>1101</td>
<td>Regenerated Cellulose - MWCO 12-14K</td>
<td>50u/pack</td>
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<tr>
<td>1105</td>
<td>Regenerated Cellulose - MWCO 10K</td>
<td>8u/pack</td>
</tr>
<tr>
<td>1104</td>
<td>Regenerated Cellulose - MWCO 25K</td>
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<tr>
<td>1150</td>
<td>Regenerated Cellulose - MWCO 50K</td>
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### Other consummables

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<th>Cat.#</th>
<th>Name</th>
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<tr>
<td>40069</td>
<td>HTD 96a/b - Adhesive Sealing Film</td>
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### APPLICATIONS
- Serum/Plasma protein binding studies, Plasma and whole blood drug partitions
- Microsome protein binding, receptor binding studies, and T4 free fraction binding (hyperthyroidism) determinations.
- Formulation of drug dosage for in vitro use, Solubility studies
- Drug-drug interaction studies
- Tissue homogenate binding studies, relative binding of drug to specific tissues

HTD system is widely used by leading Pharmaceutical and Biotechnology companies as well as Universities worldwide.

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### HTD96a: Operating Instructions

**Components**

- Teflon block assembly: 9 Teflon bars, labeled sequentially from A through I, are assembled with two stainless-steel connecting rods.
- Cam levers
- Stainless steel pressure plate

**Cleaning the Teflon bars**

1. Open the clamp by rotating the cam lever 180 degrees. To ensure even pressure, always use 2 hands to operate the clamp.
2. Remove the entire Teflon block assembly from the clamp, then remove the stainless-steel pressure plate.
3. Remove one or two Teflon bars from one end of the assembly, then pull out both stainless-steel connecting rods. (Teflon bars will disassemble.)
4. Clean separated Teflon bars with non-ionic laboratory detergent.

Note: Always rinse away all detergent residue with distilled water before re-use.
Hydrating the dialysis membranes

Each dry membrane strip consists of a pair of dialysis membranes that separate upon hydration. Hydrate the dialysis membranes by soaking in distilled H₂O or phosphate buffered saline (PBS) for 60 minutes. Add 20% by volume ethanol and soak for an additional 20 minutes. Membranes are ready for use or may be left in the 20% ethanol for extended storage. Prior to use, membranes must be rinsed twice in distilled H₂O or PBS.

Make sure only a single sheet of dialysis membrane is placed between Teflon bars.

Wipe off excessive buffer from the membranes.

Flat membrane before the next Teflon bar is put in place.

Assembling the Teflon bars with dialysis membranes

1. Lay the first Teflon bar labeled "A" Teflon on the bench. Insert the two stainless steel nailing rods so they are perpendicular to the Teflon bar.

2. Place the membrane on the Teflon bar. Ensure that the membrane is approximately 2 mm below the top edge of the bar and the lower membrane edge overlaps the bottom of all wells.

3. Repeat layering dialysis membranes and Teflon bars until the unit is fully assembled (assemble Teflon bars in alphabetical order).

Note: Although the number of membranes inserted can be varied to suit the experimental requirements, all the Teflon bars must be assembled before loading the Teflon block into the base.

Loading the Teflon block into the base

1. Make sure the clamp is in the open position before loading the Teflon block into the base.

2. Insert the Teflon block into the base, then place the stainless steel pressure plate between the Teflon block and the cam as shown.

3. Always tighten the assembled unit with even pressure by using both hands to rotate the cam lever.

Important Note:
Immediately add buffer to the dialysis side of the wells to prevent dehydration of the membranes before the test samples are added.

Unit is ready for use!

Loading samples into dialysis wells

Samples can be added to the dialysis wells using any standard 8- or 12-well multi-channel spotting device or 96-well spotting instrument. Test samples are most conveniently prepared in 96-well tubes or plates, since this facilitates preparation of dilutions, replicate samples and mixing. Volumes of 25 to 150 µl can be used in this apparatus, however it is essential that equal volumes are used on each side of the membrane.

Dialysis

After loading, cover the top surface of the Teflon block with an adhesive sealing film to prevent evaporation and maintain a constant pH. The dialysis block can be incubated at any desired temperature. Equilibrium is reached more rapidly if the block is placed on an orbital or reciprocating shaker. Although most compounds reach equilibrium in about four hours, the standard protocol recommends six hours dialysis to ensure equilibrium conditions are achieved.

Samples can be added or removed from the dialysis well at anytime during the experiment by merely removing the adhesive seal to gain access.

Care and Storage of the HTD 96a

After each use, the HTD 96a should be cleaned with a non-ionic laboratory detergent and rinsed thoroughly with distilled water. Care must be taken to ensure that all detergent has been rinsed from the unit as residual detergent could compromise future bonding studies. The Teflon block may also be disinfected with a 1% bleach solution if desired; it is essential to leave the unit in the "Open Clamp" position to dilute and remove the bleach solution. The HTD 96a unit in the closed position for extended periods may compress the Teflon and reduce the effectiveness of the base clamp.
FAQs

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 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.

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.

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 after 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.

What incubation temperatures and shakers can I use?
The dialysis block can be incubated at any desired temperature between 20°C and 45°C. 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).

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.

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.

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 4°C 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.

How long can we store membranes in 20% ethanol after hydration?
We recommend storing them no longer than 4 weeks in 20% ethanol at 4°C 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.

**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 300μl. Detection sensitivity will often dictate the appropriate volume required.

**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.

**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.

**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.

**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 37°C 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.

**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.

**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.

**What causes difficulty in closing the clamp?**

Inadvertently using double membranes in the Teflon block instead of ensuring they are separated after hydration.

**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.

**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 7.4) spiked with 10μM test compound to the Sample side of the dialysis well.
3. Dialyze for 6 hours
4. ACN precipitate and dilute samples prior to analysis in 1.2ml polypropylene tubes. (see instructions below)
   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/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.

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%

Related products/documents
Products HighLights Overview, including:
- Dialysis selection guide
- Other desalting tools: gelfiltration columns, ProteoCon
- Lab Microfiltration, Lab Macrofiltration

Information inquire

Reply by Fax: +33 (0) 4 70 03 82 60 or email at interbiotech@interchim.com

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