

Protocol for Serum Protein Binding Assay Using HTD 96*

After assembling dialysis apparatus:

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
3. Acetonitrile 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/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 = f_u = 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.

Notes

The dialysis block can be incubated at any desired temperature. Equilibrium is reached more rapidly if the dialysis block is shaken during the incubation period. This can be accomplished by placing the dialysis block in a shaking incubator or any general incubator containing an orbital or reciprocating platform shaker with a deck large enough to hold one or more dialysis

blocks depending on one's needs. Shaking at 80-100rpm is sufficient. Some of the many platform shakers available include ones marketed by Fisher Scientific (Catalog # 14-278-104) and VWR international (Catalog # 47742-750 or #33998-360).

After each use the HTD dialysis block should be cleaned thoroughly with a non-ionic detergent. We generally disassemble the dialysis block and soak overnight in a 2-liter beaker containing a 2% solution of Micro 90 (Micro 90 concentrated cleaning solution can be purchased from VWR international, Catalog # 21830-416). The Teflon bars are then rinse thoroughly with distilled water, first in the 2 liter beaker and then 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% bleach solution if desired. Never use any abrasive or brush for cleaning the Teflon blocks as they will cause micro-striations and prevent effective sealing.

*HTD 96 is a patented technology from HTDialysis, LLC