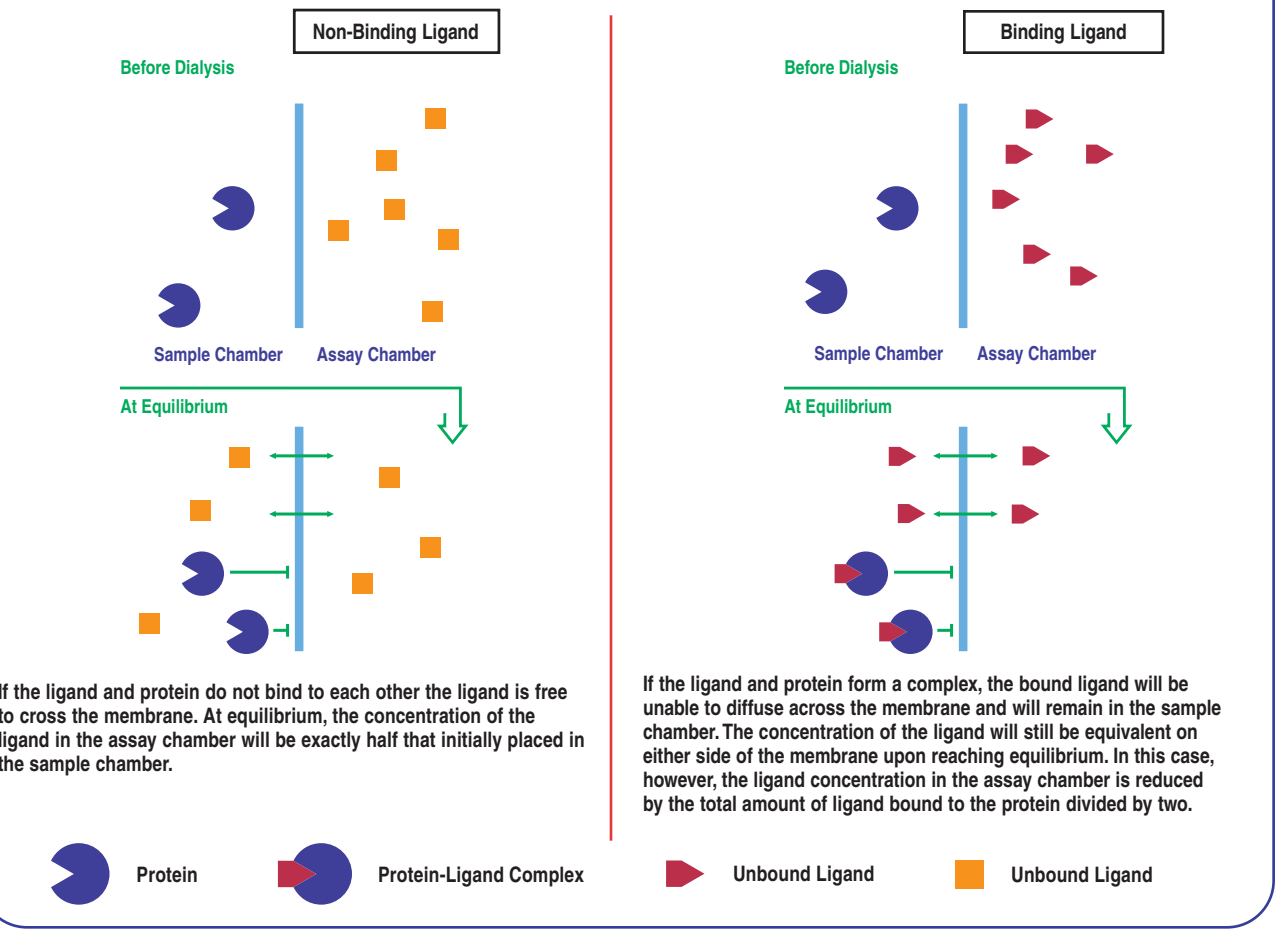
Equilibrium Dialysis

Introduction to Equilibrium Dialysis

How Does Equilibrium Dialysis Work?



If the ligand and protein do not bind to each other the ligand is free to cross the membrane. At equilibrium, the concentration of the ligand in the assay chamber will be exactly half that initially placed in the sample chamber.

If the ligand and protein form a complex, the bound ligand will be unable to diffuse across the membrane and will remain in the sample chamber. The concentration of the ligand will still be equivalent on either side of the membrane upon reaching equilibrium. In this case, however, the ligand concentration in the assay chamber is reduced by the total amount of ligand bound to the protein divided by two.

Applications:

- Protein-drug binding assays
- Receptor binding assays
- Ligand binding assays
- Protein-protein interactions
- Protein-DNA interactions
- Serum protein binding

Equilibrium dialysis is a specific application of dialysis that is important for the study of the binding of small molecules and ions by proteins. It is one of several methods available for this purpose, and its attractive feature continues to be its physical simplicity. Another attractive feature of equilibrium dialysis is the ability to perform interaction studies without the use of fluorescent or radiolabeled tags.

Generally, the objective of an equilibrium dialysis experiment is to measure the amount of a ligand bound to a macromolecule. This is typically done through an indirect process because in any mixture of the ligand and macromolecule, it is difficult to distinguish between the bound and free ligand. If, however, the free ligand can be dialyzed through a membrane, until its concentration across the membrane is at equilibrium, the free ligand concentration can be measured easily. Data obtained under different experimental conditions then provides information on various binding parameters of the compounds such as the binding constants and the number of binding sites or binding capacity.

Harvard Apparatus/AmiKa offers four types of Equilibrium DIALYZERS™. These products can meet virtually all of your bind-interaction application requirements:

Micro-Equilibrium DIALYZER™

The Micro-Equilibrium DIALYZER is available as 2- or 3-chamber systems. It is used to study interactions between biomolecules such as the binding of a ligand to a protein. For sample volumes from 25µl to 500µl, see page 399.

Multi-Equilibrium DIALYZER™

For simultaneous and highly reproducible equilibrium dialysis of up to 20 samples with volumes from 0.25 to 5ml, see page 400.

DispoEquilibrium DIALYZER™

A disposable version of the Micro-Equilibrium DIALYZER suitable for samples from 25 to 75µl, see page 401.

96-Well Equilibrium DIALYZER™

A 96-well disposable equilibrium DIALYZER for high throughput interaction studies. For samples up to 50µl to 200µl, see page 401

Contact The Nest Group, Inc. to receive a free copy of the 'Guide to Equilibrium Dialysis'.

DispoEquilibrium DIALYZER™
Samples from 25µl to 75µl

Advantages:

- Easy to use & disposable
- Small sample volumes: 25µl to 75µl each chamber
- Rapid dialysis due to ultra-thin membrane
- High-quality regenerated cellulose membranes with MWCOs of 5,000 and 10,000 Daltons
- Leak-proof

Applications:

- Protein and Protein-drug binding assays
- Receptor binding assays
- Ligand binding assays
- Protein-protein and Protein-DNA interactions

Harvard Apparatus/Amika's DispoEquilibrium DIALYZER is a single-use product for interaction studies and is currently the only such device on the market. The DispoEquilibrium DIALYZER is leak-proof and provides high sample recovery (almost 100 percent). This system is designed for one-time use with samples such as radiolabeled compounds, avoiding the hassle associated with cleaning the DIALYZER after use.

Each chamber has a capacity of 25 to 75µl. The DispoEquilibrium DIALYZER utilizes high-quality regenerated cellulose membranes with MWCO's of 5,000 or 10,000 Daltons. Sample recovery is very easy through centrifugation or via removal with micropipettes.

DispoEquilibrium DIALYZERS			
Membrane MWCO (Daltons)	Qty. of 25	Qty. of 50	Qty. of 100
5,000	SDIS 050JE	SDIS 050KE	SDIS 050ME
$	82.00	136.00	245.00
10,000	SDIS 100JE	SDIS 100KE	SDIS 100ME
$	82.00	136.00	245.00
Other MWCO are available upon request			
Extra Loading Pippette Tips, pkg. of 100			NP 74-2222
$			34.00

96-Well Equilibrium DIALYZER™
Samples from 50µl to 200µl

Key	Membrane

Advantages:

- 96-well format
- Individual membrane for each well
- Small sample volumes: 50µl to 200µl
- Ultra-thin regenerated cellulose membranes
- Membranes are free of sulfur and heavy metal contamination
- High well-to-well reproducibility
- Excellent sample recovery (>95%)

Applications:

- Protein and Protein-drug binding assays
- Receptor binding assays
- Ligand binding assays
- Protein-protein and Protein-DNA interactions

The single use, 96-Well Equilibrium DIALYZER is a novel product for the simultaneous assay of 96 samples. Each well in this system has a separate membrane and thus eliminates the possibility of sample cross-contamination. Reproducibility is very high across the different wells of the Equilibrium DIALYZER and sample recovery is excellent. Wells 96-Well are sealed with 8-cap strips. Thus a row of wells or all 96 wells can be used depending on the specifications of the experiment. The 96-Well Equilibrium DIALYZER utilizes high-quality regenerated cellulose membranes available with MWCO's of 5,000 or 10,000 Daltons.

Catalog No.	$	Description
SDIS 9605EN	383.00	96-Well Equilibrium DIALYZER Plate Membrane MWCO 5,000 Daltons, 1/pkg, ($1080.00 / 3pk)
SDIS 9610EN	383.00	96-Well Equilibrium DIALYZER Plate Membrane MWCO 10,000 Daltons, 1/pkg, ($1080.00 / 3pk)
SPLR 0000.1	710.00	Single Plate Rotator, pkg. of 1
SPLR 0008H	3050.00	8-Plate Rotator Oven, pkg. of 1
SPLR 0000	825.00	Dual Plate Rotator, pkg. of 1

Introduction to Equilibrium Dialysis

Equilibrium dialysis is a specific application of the general phenomenon of dialysis that is important for the study of the binding of small molecules and ions by proteins. It is one of several methods currently available but its attractive feature continues to be its physical simplicity.

The objective of an equilibrium dialysis experiment is usually to measure the amount of a ligand bound to a macro-molecule. This is typically done through an indirect method because in any mixture of the ligand and macro-molecule, it is difficult to distinguish between bound and free ligand. If, however, the free ligand can be dialyzed through a membrane, until its concentration across the membrane is at equilibrium, free ligand concentration $C_{L\ (f)}$ and the following data can be measured:

Temperature (absolute)	T
Concentration of binding component, e.g. protein	$C_{P\ (o)}$
Starting concentration of ligand	$C_{L\ (o)}$
Final concentration of free ligand	$C_{L\ (f)}$

From which the following parameters can be derived directly:

Concentration of bound ligand	$C_{L(b)}$
Free fraction (of ligand)	f
Bound fraction (of ligand)	b
Degree of binding or saturation fraction	r

Data obtained from several experiments at a range of temperatures and with varying initial concentration of ligand can provide other binding parameters:

Association constant	K
Number of binding sites	n
Binding capacity	N

Further, the thermodynamics of the binding reaction can be derived:

Change of free energy	ΔG
Enthalpy change	ΔH
Entropy change	ΔS

Since equilibrium exists, the value $C_{L\ (f)}$ is the same on both sides of the membrane. *(Note: where charged species are involved the Gibbs-Donnan effect can upset the equilibrium unless moderately concentrated salts are in solution; say 0.6% NaCl).*

Hence: $$C_{L\ (o)} = C_{L\ (f)} + C_{L\ (f)} + C_{L\ (b)}*$$

**It is essential to correct this equation to take account of any ligand which might be bound to the membrane.*

$$C_{L\ (b)} = C_{L\ (o)} - 2 \times C_{L\ (f)}$$

The free fraction f is given by:

$$f = \frac{C_{L\ (f)}}{C_{L\ (o)} - C_{L\ (f)}}$$

The bound fraction b is: $b = 1 - f$

The degree of binding or saturation fraction r is:

$$r = \frac{C_{L\ (b)}}{C_{P\ (o)}}$$

If the protein concentration is known, the Scatched plot can be used to determine binding constants and the number of binding sites. If the protein concentration is unknown, the absolute number of binding sites is replaced by binding capacity N.

In the former case, values of r would be plotted on the abscissa against $r/C_{L\ (f)}$ on the ordinate. If only one class of binding sites is present, the Scatched plot results in a straight line with slope equal to $-K$ see Fig. 1.

The intercept on the abscissa give the value n. If two classes of binding sites are involved, the plot takes the form of an hyberbola. In this case, the asymptotes have slopes equal to $-K$ for each class of site, and their intercepts on the abscissa give the two values for n. The intercept between the curve and the abscissa is equal to the sum of the two values for n, see Fig. 2.

The free energy change is obtained simply by substituting the appropriate values in the following equation:

$$\Delta G = -RT\ln K$$

Where R is the gas constant.

ΔH can be obtained from a graph based upon an integrated form of the van/t Hoff equation.

$$\ln K = \frac{-\Delta H}{RT} + C$$

In this case a plot $\ln K$ versus $1/T$ has a slope of $-\Delta H/R$. Once a value for ΔH has been found it can be substituted into: $\Delta G = sH - T\Delta S$ to obtain a result for the entropy change ΔS.

Fig. 1.

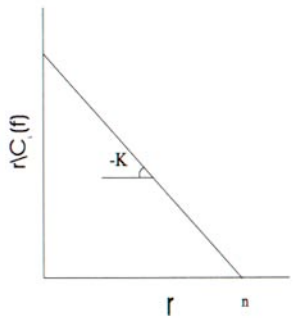
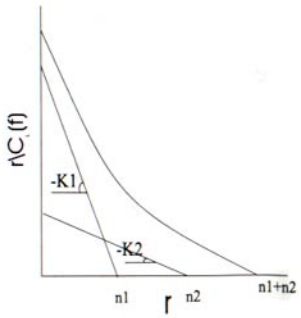


Fig. 2.



Scatchard, G., Am. N.Y. Acad. Sci. ***51****, 660-672 (1949)*
Rosenthal, H.E., Anal. Biochem. ***20****, 525-532 (1967)*
Weder, H.G., Schildknecht, J., Lutz, R.A. and Kesselring, P., Eur. L. Biochem. ***42****, 475-481 (1974)*
AmiKa NEWS, Vol. V111, (1999)

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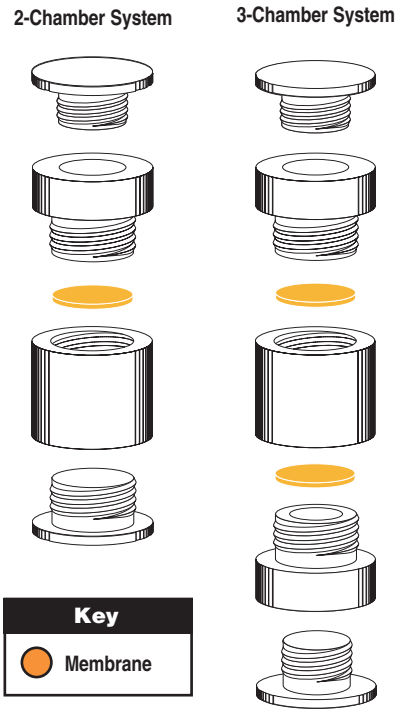
MicroEquilibrium DIALYZER - Samples from 25µl to 500µl (Reusable)



The binding and ligand elements are placed in one chamber (the sample chamber) while the other chamber (the assay chamber) contains an equivalent volume of the same buffer without either element. When equilibrium has been reached the concentration of the ligand in the assay chamber can be measured and analyzed to obtain the results of the assay.
When the ligand is free in solution it can readily pass through the membrane, but when complexed, it is too large and is retained by the membrane.

For Membrane ordering information, see pages 402-404

Membrane ordering information is color coded to assist you in selecting the appropriate membrane:

Pink shaded membranes are for products with sample sizes up to 200µl

Purple shaded membranes are for products with sample sizes ranging from 250µl to 1,500µl

Green shaded membranes are for products with sample sizes ranging from 3,000µl to 5,000µl

Advantages:

- Easy to use
- Leak-proof
- Reusable
- Available for a range of sample sizes
- Membranes available with MWCO's to suit almost any application
- Autoclaveable
- Low protein binding
- High sample recovery
- Made of Teflon – totally inert

The Micro-Equilibrium DIALYZER is a unique equilibrium dialysis chamber for small samples (25 to 500µl). Due to the small volume of the chamber, very small amounts of sample are required for protein binding assays. Two chambers of equivalent volume are joined together with a membrane between them, as shown. When dialysis is complete the chambers can be opened at each end to extract the sample for analysis. The entire system can also be placed in a thermostat for temperature-controlled dialysis.

Applications:

- Protein binding assays
- Protein-drug binding assays
- Receptor binding assays
- Ligand binding assays
- Protein-protein interations
- Protein-DNA interactions

The Micro-Equilibrium DIALYZER can also be used with three chambers instead of two. One of the main advantages of using this configuration is that the results can be obtained without waiting for equilibrium to be reached, thus reducing the assay time.

This is achieved by placing the assay compound in the central chamber; the binding component in one of the terminal chambers and control buffer, containing neither component, in the remaining chamber. Comparing the concentration of the assay compound in the two terminal chambers will then yield information on the binding characteristics of the assay components.

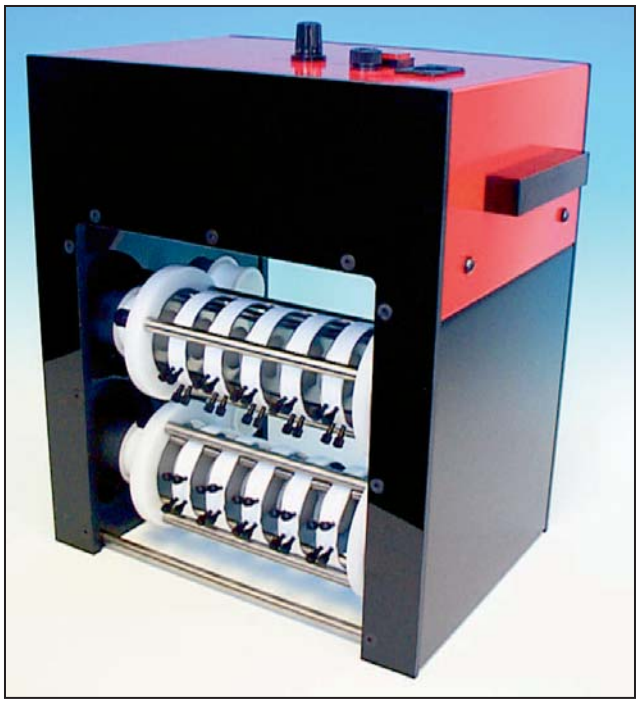
Micro-Equilibrium DIALYZERS

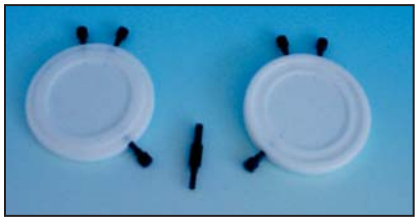
Volume per Chamber (µl)	Total Volume (µl)	Qty. of 1	$	Qty. of 5	$
25	50	SSE 0050.1	136.00	SSE 0050	546.00
50	100	SSE 0100.1	136.00	SSE 0100	546.00
100	200	SSE 0200.1	136.00	SSE 0200	546.00
250	500	SSE 0500.1	136.00	SSE 0500	546.00
500	1,000	SSE 1000.1	136.00	SSE 1000	546.00
Additional Chambers for 3-Chamber System					
25	–	SER 0025.1	71.00	SER 0025	271.00
50	–	SER 0050.1	71.00	SER 0050	271.00
100	–	SER 0100.1	71.00	SER 0100	271.00
250	–	SER 0250.1	71.00	SER 025	271.00
500	–	SER 0500.1	71.00	SER 0500	271.00

Ultra-Thin Membranes for Micro-Equilibrium DIALYZERS

Membrane MWCO (Daltons)	Qty. of 24	$	Qty. of 96	$
For Use with 25µl, 50µl and 100µl Volume Chambers				
5,000	SA 050S.24	44.00	SA 050S	139.00
10,000	SA 100S.24	44.00	SA 100S	139.00
For Use with 250µl and 500µl Volume Chambers				
5,000	SB 050D.24	54.00	SB 050D	195.00
10,000	SB 100D.24	54.00	SB 100D	195.00

Multi-Equilibrium DIALYZER™ - Samples from 0.25ml to 5ml (Reusable)





Advantages:

- Easy to use
- Leak-proof
- Reproducible
- Fast dialysis times
- Available for a range of sample sizes
- Up to 20 parallel, simultaneous assays
- Autoclavable
- Low protein binding
- High sample recovery
- Made of Teflon – totally inert

Applications:

- Protein binding assays
- Protein-drug binding assays
- Receptor binding assays
- Ligand binding assays
- Protein-protein interations
- Protein-DNA interactions

The Harvard Apparatus/Amika Multi-Equilibrium DIALYZER provides highly standardized equilibrium dialysis conditions for up to 20 parallel assays. The instrument offers outstanding uniformity of:

- Membrane Area
- Sample Volume
- Degree of Agitation

The advantages of this system are that up to 20 cells can be used simultaneously for rapid dialysis under standardized conditions. Experiments conducted using the Multi-Equilibrium DIALYZER are extremely reproducible and leak-proof and can be performed at a constant temperature.

The DIALYZER cells are made of Teflon, an extremely inert material, and will not interfere with the samples. Multiple cell systems are available (5, 10, 15, 20 cells) at various cell volumes (0.25, 1.0, 2.0 & 5.0 ml). The unit can be sterilized by autoclaving and the cells can be filled easily with a filling clamp.

Multi-Equilibrium DIALYZER Systems

Catalog No.	$	Description
NP 74-1800	8,044.00	Complete Multi-Equilibrium DIALYZER System • Ready-to-Use Teflon Macro Dialysis Cells (1ml) with Large Surface Area and 20 plugs, pkg. of 20 • Variable Speed Drive Unit for 20 Cells, pkg. of 1 (12" x 12" x 7.5") • Stand, pkg. of 1 • Filling Clamp, pkg. of 1 • Carriers for 5 Teflon Dialysis Cells, pkg. of 4 • Emptying Stoppers, pkg. of 20 • Dialysis Membranes, MWCO 10,000 Daltons with Very High Permeability, pkg. of 200

Membranes for Multi-Equilibrium DIALYZER

Catalog No.	$	Description
NP 74-2100	191.00	MWCO 5,000 Daltons, pkg. of 200
NP 74-2102	213.00	MWCO 10,000 Daltons with Very High Permeability, pkg. of 200

Multi-Equilibrium DIALYZER Individual Components

Catalog No.	$	Description
NP 74-1901	94.00	Emptying Stoppers, pkg. of 5
NP 74-1903	1,256.00	Macro Teflon Dialysis Cells (1ml), pkg. of 5
NP 74-1904	1,256.00	Macro Teflon Dialysis Cells (2ml), pkg. of 5
NP 74-1905	1,256.00	Macro Teflon Dialysis Cells (5ml), pkg. of 5
NP 74-1906	1,256.00	Macro Teflon Dialysis Cells with 5 Large Surface Area (1ml)
NP 74-1907	1,256.00	Micro Teflon Dialysis Cells (0.2ml), pkg. of 5
NP 74-1908	82.00	Spacer, Micro, pkg. of 1
NP 74-1909	55.00	Spacer, Macro, pkg. of 1
NP 74-1910	825.00	Cell Carrier, Micro, pkg. of 1
NP 74-1911	546.00	Cell Carrier, Macro, pkg. of 1
NP 74-1912	166.00	Power Supply Adapter (110 V)
NP 74-1912A	166.00	Power Supply Adapter (220 V)
NP 74-1913	281.00	Filling Clamp, pkg. of 1
NP 74-1914	166.00	Black Stoppers, pkg. of 32
NP 74-1919	213.00	Tank w/ Fittings, pkg. of 1