

# Guide to Equilibrium Dialysis

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# Introduction

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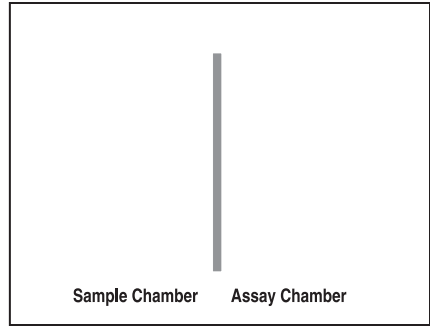
Equilibrium Dialysis is a simple but effective tool for the study of interactions between molecules. Whether it be characterization of a candidate drug in serum binding assays or detailed study of antigen-antibody interactions, equilibrium dialysis proves to be the most accurate method available. Equilibrium dialysis is inexpensive and easy to perform, the only instrumentation required is that used to quantify the compound of interest. Since the results of the assay are obtained under equilibrium conditions, the true nature of the interaction can be studied. Equilibrium dialysis also offers the ability to study low affinity interactions that are undetectable using other methods.

This guide offers an introduction to the technique of equilibrium dialysis and some examples of how this technique can be used in real world applications. There is also an introduction to the types of data analysis methods used to extract results from these types of experiments. Details of the wide range of equilibrium dialysis products offered by Harvard Apparatus can be found towards the back of this booklet.

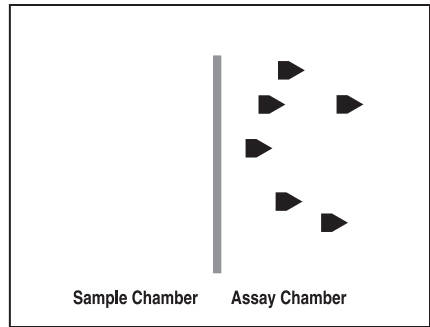
# Protocol

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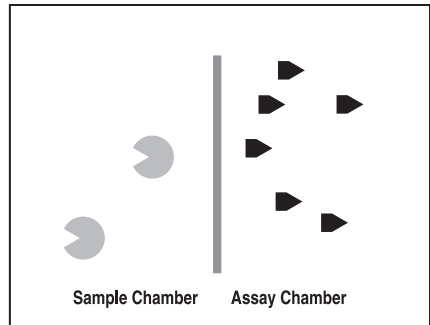
In a standard equilibrium dialysis assay you begin with two chambers separated by a dialysis membrane. The molecular weight cut off (MWCO) of this membrane is chosen such that it will retain the receptor component of the sample (the element which will bind the ligand).



A known concentration and volume of ligand is placed into one of the chambers. The ligand is small enough to pass freely through the membrane.

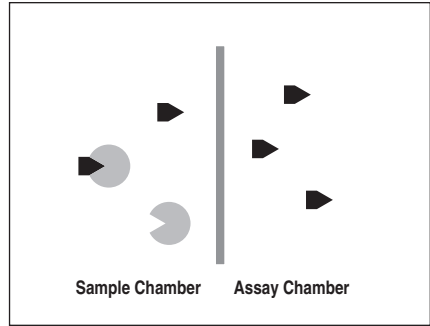


A known concentration of receptor is then placed in the remaining chamber in an equivalent volume to that placed in the first chamber.

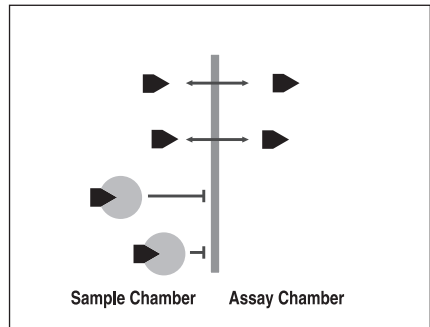


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As the ligand diffuses across the membrane some of it will bind to the receptor and some will remain free in solution. The higher the affinity of the interaction, the higher the concentration of ligand that will be bound at any time.



Diffusion of the ligand across the membrane and binding of the ligand continues until equilibrium has been reached. At equilibrium, the concentration of ligand free in solution is the same in both chambers. In the receptor chamber, however, the overall concentration is higher due to the bound-ligand component.



The concentration of free ligand in the ligand chamber can then be used to determine the binding characteristics of the samples as described in the next section.

# Data Analysis

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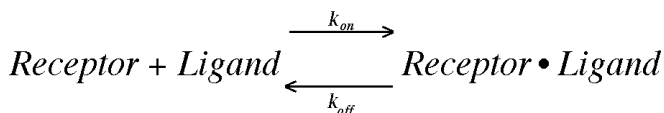
Equilibrium Dialysis can be used in a wide variety of experiments and the methods used to analyze the resulting data can vary just as widely. This section serves as an introduction to the types of data analysis tools used to interpret experimental data generated using equilibrium dialysis.

The type of assay typically performed using equilibrium dialysis falls under the category of saturation binding experiments. In this case the equilibrium binding of various concentrations of the receptor and ligand is measured. The relationship between binding and ligand concentration is then used to determine the number of binding sites,  $B_{max}$ , and the ligand affinity,  $K_d$ . Because this kind of experimental data used to be analyzed with Scatchard plots, they are sometimes called “Scatchard experiments”.

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## Analysis of Ligand Binding Data

Analysis of ligand binding experiments is based on a simple model, called the law of mass action. This model assumes that binding is reversible.



Binding occurs when ligand and receptor collide due to diffusion, and when the collision has the correct orientation and enough energy. The rate of association is:

$$[\text{Ligand}] \cdot [\text{Receptor}] \cdot k_{on}$$

[ ] denotes concentration

The association rate constant ( $k_{on}$ ) is expressed in units of  $M^{-1}min^{-1}$ . Once binding has occurred, the ligand and receptor remain bound together for a random amount of time. The probability of dissociation is the same at every instant of time. The receptor doesn't "know" how long it has been bound to the ligand. The rate of dissociation is:

$$[\text{Ligand} \cdot \text{Receptor}] \cdot k_{off}$$

The dissociation constant  $k_{off}$  is expressed in units of  $min^{-1}$ . After dissociation, the ligand and receptor are the same as at they were before binding. If either the ligand or receptor is chemically modified, then the binding does not follow the law of mass action. Equilibrium is reached when the rate at which new ligand-receptor complexes are formed equals the rate at which the ligand-receptor complexes dissociate. At equilibrium:

$$[\text{Ligand}] \cdot [\text{Receptor}] \cdot k_{on} = [\text{Ligand} \cdot \text{Receptor}] \cdot k_{off}$$

Rearrange that equation to define the equilibrium dissociation constant  $k_d$ . Define the equilibrium dissociation constant,  $k_d$  to equal  $k_{off}/k_{on}$ , which is in molar units. In enzyme kinetics, this is called the Michaelis-Menten constant,  $K_M$ .

$$\frac{[Ligand] \cdot [Receptor]}{[Ligand \cdot Receptor]} = \frac{k_{off}}{k_{on}} = k_d$$

The  $k_d$  has a meaning that is easy to understand. Set  $[Ligand]$  equal to  $k_d$  in the equation above. The  $k_d$  terms cancel out, and you'll see that  $[Receptor]/[Ligand \cdot Receptor]=1$ , so  $[Receptor]$  equals  $[Ligand \cdot Receptor]$ . Since all the receptors are either free or bound to ligand, this means that half the receptors are free and half are bound to ligand. In other words, when the concentration of ligand equals the  $k_d$ , half the receptors will be occupied at equilibrium. If the receptors have a high affinity for the ligand, the  $k_d$  will be low, as it will take a low concentration of ligand to bind half the receptors.

Don't mix up  $k_d$ , the equilibrium dissociation constant, with  $k_{off}$ , the dissociation rate constant. They are not the same, and aren't even expressed in the same units.

Variable	Name	Units
$k_{on}$	Association rate constant or on-rate constant	$M^{-1}min^{-1}$
$k_{off}$	Dissociation rate constant or off-rate constant	$min^{-1}$
$k_d$	Equilibrium dissociation constant	$M$



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Fractional occupancy is the fraction of all receptors that are bound to ligand.

$$\text{Fractional Occupancy} = \frac{[\text{Ligand} \cdot \text{Receptor}]}{[\text{Total Receptor}]} = \frac{[\text{Ligand} \cdot \text{Receptor}]}{[\text{Receptor}] + [\text{Ligand} \cdot \text{Receptor}]}$$

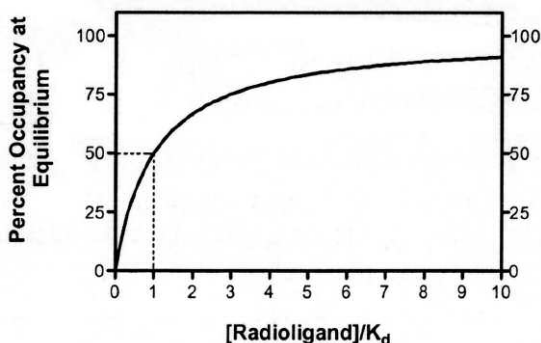
This equation can be more clearly represented as:

$$\text{Fractional Occupancy} = \frac{[\text{Ligand}]}{[\text{Ligand}] + K_d}$$

This equation assumes equilibrium. To make sense of it, think about a few different values for [Ligand].

[Ligand]	Fractional Occupancy
0	0
$1 \cdot k_d$	50%
$4 \cdot k_d$	80%
$9 \cdot k_d$	90%
$99 \cdot k_d$	99%

This becomes even clearer in graphical form.



Note that when [Ligand]=k<sub>d</sub>, fractional occupancy is 50%.

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Although termed a “law”, the law of mass action is simply a model that can be used to explain some experimental data. Because it is so simple, the model is not useful in all situations. The model assumes:

- All receptors are equally accessible to ligands.
- Receptors are either free or bound to ligand. It doesn't allow for more than one affinity state, or states of partial binding.
- Binding does not alter the ligand or receptor.
- Binding is reversible.

Despite its simplicity, the law of mass action has proven to be very useful in describing many aspects of receptor pharmacology and physiology.

# Linear Regression

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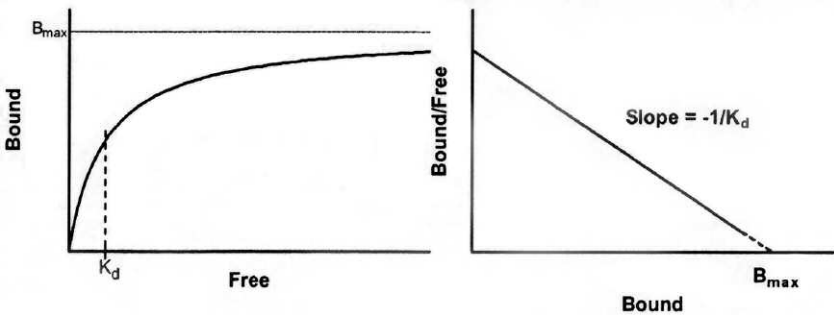
## Linear Regression: Introduction

In the days before nonlinear regression programs (eg. Graphpad Prism®) were widely available, scientists transformed data into a linear form, and then analyzed the data by linear regression.

Linear regression analyzes the relationship between two variables, X and Y. For each subject (or experimental unit), you know both X and Y and you want to find the best straight line through the data. In some situations, the slope and/or intercept have a scientific meaning. In other cases, you use the linear regression line as a standard curve to find new values of X from Y, or Y from X. In general, the goal of linear regression is to find the line that best predicts Y from X. Linear regression does this by finding the line that minimizes the sum of the squares of the vertical distances of the points from the line.

## Linear Regression: The Scatchard Plot

There are several ways to linearize binding data, including the methods of Lineweaver-Burke and Eadie-Hofstee. However, the most popular method to linearize binding data is to create a Scatchard plot, as shown in the right panel below.



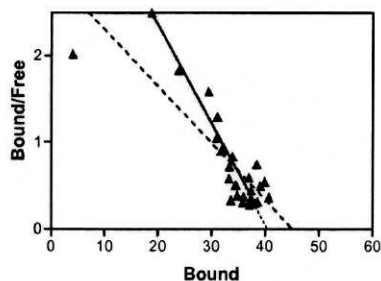
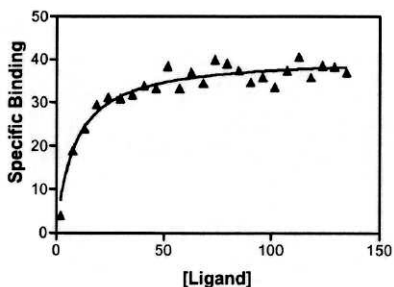
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In this plot, the X-axis is specific binding and the Y-axis is specific binding divided by free ligand concentration. It is possible to estimate the  $B_{max}$  and  $k_d$  from a Scatchard plot ( $B_{max}$  is the X intercept;  $k_d$  is the negative reciprocal of the slope). However, the Scatchard transformation distorts the experimental error, and thus violates several assumptions of linear regression. The  $B_{max}$  and  $k_d$  values you determine by linear regression of Scatchard transformed data may be far from their true values.

### Linear Regression: *Analysis*

The problem with this method is that the transformation distorts the experimental error. Linear regression assumes that the scatter of points around the line follows a Gaussian distribution and that the standard deviation is the same at every value of X. These assumptions are rarely true after transforming data. Furthermore, some transformations alter the relationship between X and Y. For example, in a Scatchard plot the value of X (bound) is used to calculate Y (bound/free), and this violates the assumption of linear regression that all uncertainty is in Y while X is known precisely. It doesn't make sense to minimize the sum of squares of the vertical distances of points from the line, if the same experimental error appears in both X and Y directions. Since the assumptions of linear regression are violated, the values derived from the slope and intercept of the regression line are not the most accurate determinations of the variables in the model. Considering all the time and effort you put into collecting data, you want to use the best possible technique for analyzing your data. Nonlinear regression produces the most accurate results.

The graph below shows the problem of transforming data. The left panel shows data that follows a rectangular hyperbola (binding isotherm). The right panel is a Scatchard plot of the same data. The solid curve on the left was determined by nonlinear regression. The solid line on the right shows how that same curve would look after a Scatchard transformation. The dotted line shows the linear regression fit of the transformed data. Scatchard plots can be used to determine the receptor number ( $B_{max}$ , determined as the X-intercept of the linear regression line) and dissociation constant ( $k_d$ , determined as the negative reciprocal of the slope). Since the Scatchard transformation amplified and distorted the scatter, the linear regression fit does not yield the most accurate values for  $B_{max}$  and  $k_d$ .



Don't use linear regression just to avoid using nonlinear regression. Fitting curves with nonlinear regression is not difficult using software programs such as Graphpad Prism<sup>®</sup>. Although it is usually inappropriate to analyze transformed data, it is often helpful to display data after a linear transform. Many people find it easier to visually interpret transformed data. Even if you analyze your data with nonlinear regression, it may make sense to display the results of a linear transform.

# Non-Linear Regression

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## Non-Linear Regression: *Introduction*

Linear regression is described in every statistics book, and is performed by every statistics program. Nonlinear regression is mentioned in only a few books, and is not performed by all statistics programs. From a mathematician's point of view, the two procedures are vastly different. From a scientist's point of view, however, the two procedures are very similar. In many fields of science, nonlinear regression is used far more often than linear regression. A line is described by a simple equation that calculates  $Y$  from  $X$ , slope and intercept. The purpose of linear regression is to find values for the slope and intercept that define the line that comes closest to the data. More precisely, it finds the line that minimizes the sum of the square of the vertical distances of the points from the line. The equations used to do this can be derived with no more than high-school algebra (shown in many statistics books). Put the data in, and the answers come out. There is no chance for ambiguity. You could even do the calculations by hand, if you wanted to.

Nonlinear regression is more general than linear regression. It fits data to any equation that defines  $Y$  as a function of  $X$  and one or more parameters. It finds the values of those parameters that generate the curve that comes closest to the data. More precisely, nonlinear regression finds the values of the parameters that generates a curve that minimizes the sum of the squares of the vertical distances of the data points from the curve.

Except for a few special cases, it is not possible to directly derive an equation to compute the best-fit values from the data. Instead nonlinear regression requires a computationally intensive, iterative approach. You can't really follow the mathematics of nonlinear regression unless you are familiar with matrix algebra. But these complexities only pertain to performing the calculations, which can be performed easily with non-linear regression software (like Graphpad Prism®). Using nonlinear regression to analyze data is only slightly more difficult than using linear regression. Your choice of linear or nonlinear regression should be based on the model you are fitting. Don't use linear regression just to avoid using nonlinear regression.

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## Non-Linear Regression: *Sum-of-Squares*

The goal of nonlinear regression is to adjust the values of the variables in the model to find the curve that best predicts  $Y$  from  $X$ . More precisely, the goal of regression is to minimize the sum of the squares of the vertical distances of the points from the curve. Why minimize the sum of the squares of the distances? Why not simply minimize the sum of the actual distances?

If the random scatter follows a Gaussian distribution, it is far more likely to have two medium size deviations (say 5 units each) than to have one small deviation (1 unit) and one large (9 units). A procedure that minimized the sum of the absolute value of the distances would have no preference over a curve that was 5 units away from two points and one that was 1 unit away from one point and 9 units from another. The sum of the distances (more precisely, the sum of the absolute value of the distances) is 10 units in each case. A procedure that minimizes the sum of the squares of the distances prefers to be 5 units away from two points (sum-of-squares = 25) rather than 1 unit away from one point and 9 units away from another (sum-of-squares = 82). If the scatter is Gaussian (or nearly so), the curve determined by minimizing the sum-of-squares is most likely to be correct.

## Non-Linear Regression: *Analysis*

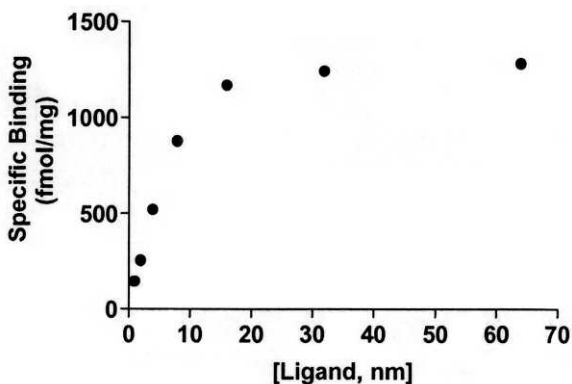
While the mathematical details of non-linear regression are quite complicated, the basic idea is pretty easy to understand. Every nonlinear regression method follows these steps:

1. Start with an initial estimated value for each variable in the equation.
2. Generate the curve defined by the initial values. Calculate the sum-of-squares (the sum of the squares of the vertical distances of the points from the curve).
3. Adjust the variables to make the curve come closer to the data points. There are several algorithms for adjusting the variables, as explained below.
4. Adjust the variables again so that the curve comes even closer to the points. Repeat.

- 
5. Stop the calculations when the adjustments make virtually no difference in the sum-of-squares.
  6. Report the best-fit results. The precise values you obtain will depend in part on the initial values chosen in step 1 and the stopping criteria of step 5. This means that repeat analyses of the same data will not always give exactly the same results.

Step 3 is the only difficult one. Prism (and most other nonlinear regression programs) uses the method of Marquardt and Levenberg, which blends two other methods, the method of linear descent and the method of Gauss-Newton.

The best way to understand these methods is to follow an example. Here are some data to be fit to a typical binding curve (rectangular hyperbola).



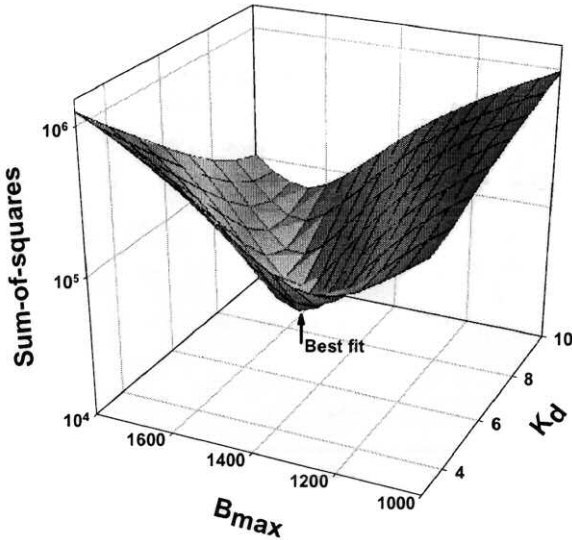
You want to fit a binding curve to determine  $B_{max}$  and  $k_d$  using the equation:

$$Y = \frac{B_{max} \cdot X}{k_d + X}$$



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How can you find the values of  $B_{max}$  and  $k_d$  that fit the data best? You can generate an infinite number of curves by varying  $B_{max}$  and  $k_d$ . For each of the generated curves, you can compute the sum-of-squares to assess how well that curve fits the data. The following graph illustrates the situation.



The X- and Y-axes correspond to two variables to be fit by nonlinear regression ( $B_{max}$  and  $k_d$  in this example). The Z-axis is the sum-of-squares. Each point on the surface corresponds to one possible curve. The goal of nonlinear regression is to find the values of  $B_{max}$  and  $k_d$  that make the sum-of-squares as small as possible (to find the bottom of the valley).

The method of linear descent follows a very simple strategy. Starting from the initial values try increasing each parameter a small amount. If the sum-of-squares goes down, continue. If the sum-of-squares goes up, go back and decrease the value of the parameter instead. You've taken a step down the surface. Repeat many times. Each step will usually reduce the sum-of-squares. If the sum-of-squares goes up instead, the step must have been so large that you went past the bottom and back up the other side. If this happens, go back and take a smaller step. After repeating these steps many times, you'll reach the bottom.

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The Gauss-Newton method is a bit harder to understand. As with the method of linear descent, start by computing how much the sum-of-squares changes when you make a small change in the value of each parameter.

This tells you the slope of the sum-of-squares surface at the point defined by the initial values. If the equation really were linear, this is enough information to determine the shape of the entire sum-of-squares surface, and thus calculate the best-fit values of  $B_{\max}$  and  $k_d$  in one step. With a linear equation, knowing the slope at one point tells you everything you need to know about the surface, and you can find the minimum in one step. With nonlinear equations, the Gauss-Newton method won't find the best-fit values in one step, but that step usually improves the fit. After repeating many iterations, you reach the bottom.

This method of linear descent tends to work well for early iterations, but works slowly when it gets close to the best-fit values (and the surface is nearly flat). In contrast, the Gauss-Newton method tends to work badly in early iterations, but works very well in later iterations. The two methods are blended in the method of Marquardt (also called the Levenberg-Marquardt method). It uses the method of linear descent in early iterations and then gradually switches to the Gauss-Newton approach. Graphpad Prism<sup>®</sup>, like most programs, uses the Marquardt method for performing nonlinear regression.

# Example

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The following example is one possible method of analysis for data from a ligand binding experiment.

In this experiment, 1ml samples of a 50,000 Da Protein (5.0 mg/ml) are allowed to come to equilibrium with 1ml volumes of a ligand solution of several concentrations. The concentrations of the ligand solutions used in the experiment are shown in the table below ( $[\text{Ligand}]_{\text{total}}$ ).

$[\text{Ligand}]_{\text{total}}$ (mmol)	$[\text{Ligand}]_{\text{free}}$ (mmol)	$[\text{Ligand}]_{\text{bound}}$ (mmol)
0.01	0.005	0.005
0.02	0.011	0.009
0.05	0.030	0.020
0.08	0.046	0.029
0.10	0.062	0.038
0.15	0.104	0.046
0.20	0.143	0.057
0.40	0.332	0.068
0.70	0.623	0.077
1.00	0.922	0.078
1.25	1.170	0.080

Once equilibrium has been reached the concentration of free ligand is measured ( $[\text{Ligand}]_{\text{free}}$ ) and the concentration of bound ligand can be determined ( $[\text{Ligand}]_{\text{bound}}$ ). The experimental results for this example are presented in the table above.

At this stage in the experiment a decision must be made regarding how the experimental data will be analyzed. In this case we will plot a binding isotherm of the data, use non-linear regression to find the best-fit line for this data (and hence determine  $B_{\text{max}}$  and  $K_d$ ). For ease of visual interpretation we will then perform a Scatchard transformation on the resultant best-fit line data.

Generating a binding isotherm for this data involves plotting ligand concentration ( $[\text{Ligand}]_{\text{free}}$ ) in millimoles on the X-axis against binding coefficient (B) on the Y-axis. The binding coefficient is given by:

$$B = \frac{[\text{Ligand}]_{\text{bound}}}{[\text{Protein}]_{\text{total}}}$$

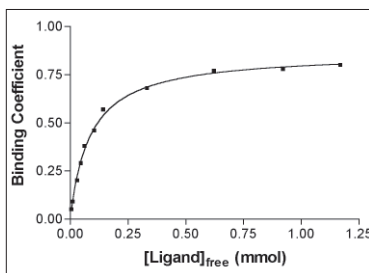
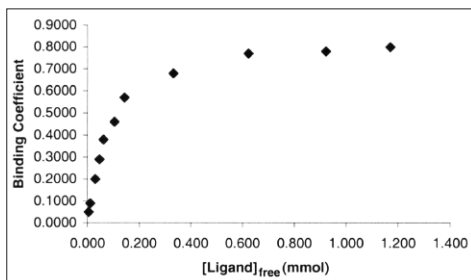
The concentration of protein is the same in each case, 0.1 mmol.

[Ligand] <sub>free</sub> (mmol)	Binding Coefficient
0.005	0.0500
0.011	0.0900
0.030	0.2000
0.046	0.2900
0.062	0.3800
0.104	0.4600
0.143	0.5700
0.332	0.6800
0.623	0.7700
0.922	0.7800
1.170	0.8000

We then use non-linear regression (Graphpad Prism®) to find the best-fit line for the data.

This can then be plotted:

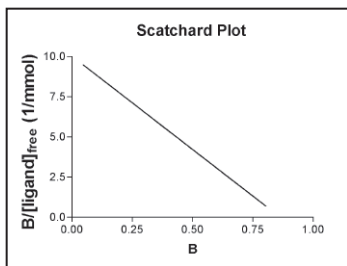
Binding Isotherm



When using a software package such as Prism, B<sub>max</sub> and K<sub>d</sub> are determined automatically. When this facility is not available it is possible to determine these values from a Scatchard plot, although this will be less accurate (as discussed in the linear regression section). The data obtained from the non-linear regression can be put through a Scatchard transformation to generate a linear plot.

The equation of this line is given by:

$$y = -11.61x + 10.03$$



The Scatchard equation is:

$$B/L = n/Kd - B/Kd$$

Where:

$$B = [\text{Ligand}]_{\text{bound}}/[\text{Protein}]_{\text{total}}$$

$$L = [\text{Ligand}]_{\text{free}}$$

$n$  = number of ligands/macromolecule, i.e. the stoichiometry

$Kd$  = the dissociation constant

Thus  $K_d$  can be determined as the negative reciprocal of the slope of the line and  $B_{\text{max}}$  is given by the X-intercept.

In this case  $Kd$  is 0.086 mmol ( $8.6 \times 10^{-5}M$ ) and  $B_{\text{max}}$  is 0.864.

## Additional Reading

For literature references on our products and to learn more about how nonlinear regression works, we recommend reading:

### DispoEquilibrium DIALYZER™

Modulating Acetyl-CoA Binding in the GCN5 Family of Histone Acetyltransferases. Langer M.R., Fry C.J., Peterson C.L. and Denu J.M., **The Journal of Biological Chemistry**, Vol. 277, No. 30, pp. 27337-27344, 2002

Three-dimensional Structure of Guanylyl Cyclase Activating Protein-2, a Calcium-sensitive Modulator of Photoreceptor Guanylyl Cyclases. Ames J.B., Dizhoor A.M., Ikura M., Palczewski K. and Stryer L., **The Journal of Biological Chemistry**, Vol. 274, No. 27, pp. 19329-19337, 1999

### Micro-Equilibrium DIALYZER™

Differential ATP Binding and Intrinsic ATP Hydrolysis by Amino-terminal Domains of the Yeast Mlh1 and Pms1 Proteins. Hall M.C., Shcherbakova P.V. and Kunkel T.A., **The Journal of Biological Chemistry**, Vol. 277, No. 5, pp. 3673-3679, 2002

### 96-Well Equilibrium DIALYZER™

Development of a High Throughput Equilibrium Dialysis Method. Kariv I., Cao H. and Oldengurg K., **Journal of Pharmaceutical Sciences**, Vol. 90, No.5, 580-587, 2001

### Multi-Equilibrium DIALYZER™

Simultaneous determination of glipizide and rosiglitazone unbound drug concentrations in plasma by equilibrium dialysis and liquid chromatography-tandem mass spectrometry. Lin Z.J., Desai-Krieger D. and Shum L., **Journal of Chromatography B**, Vol. 801, pp. 265-272, 2004

Analyzing Data with Graphpad Prism®, H.J. Motulsky, GraphPad Software, 1999. Available at [www.graphpad.com](http://www.graphpad.com)

Chapter 15 of Numerical Recipes in C, Second Edition, WH Press, et. AL., Cambridge Press, 1992.

Chapter 10 of Primer of Applied Regression and Analysis of Variance, SA Glantz and BK Slinker, McGraw-Hill, 1990.

# Products

## DispoEquilibrium DIALYZER™

Harvard Apparatus' DispoEquilibrium DIALYZER™ is a single-use product for interaction studies. 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 up to 75  $\mu$ l. 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.



### APPLICATIONS

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

### ADVANTAGES

- Easy to use
- Disposable - no clean up
- Small sample volumes:  
25 to 75  $\mu$ l each chamber
- Rapid dialysis due to ultra-thin membrane
- Membrane MWCOs of 5K and 10K Daltons
- High-quality regenerated cellulose membranes
- Leak-proof

#### DispoEquilibrium DIALYZER™

##### Membrane

MWCO (Daltons)	Qty. of 25	Qty. of 50	Qty. of 100
5,000	74-2204	74-2200	74-2201
10,000	74-2205	74-2202	74-2203

##### Catalog No.

74-2222

##### Description

Pipette Tips for Loading/Unloading

##### Quantity

100

# Products

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## Fast Micro-Equilibrium DIALYZER™

The Fast 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 on the following page. 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.

The receptor element is placed in one chamber (the sample chamber) while the other chamber (the assay chamber) contains an equivalent volume of ligand solution. 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 it is complexed it is too large and is retained by the membrane.

### APPLICATIONS

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

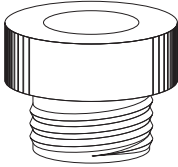
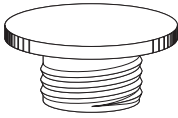
### ADVANTAGES

- 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

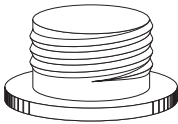
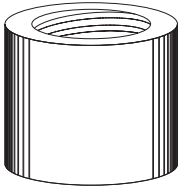
# Products

## Fast Micro-Equilibrium DIALYZER™ (Continued)

### 2-Chamber System



Membrane →



### Fast Micro-Equilibrium Dialyzers

Chamber Volume	Pack of 1	Pack of 5
25 µl	7416-251D	7416-255D
50 µl	7416-501D	7416-505D
100 µl	7416-1001D	7416-1005D
250 µl	7416-2501D	7416-2505D
500 µl	7416-5001D	7416-5005D

### Link (Additional) Chambers

25 µl	7416-251L	7416-255L
50 µl	7416-501L	7416-505L
100 µl	7416-1001L	7416-1005L
250 µl	7416-2501L	7416-2505L
500 µl	7416-5001L	7416-5005L

### Fast Micro-Equilibrium Dialyzer Membranes (pack of 25)

Regenerated Cellulose Membranes	Chamber Volume	
	25 µl, 50 µl & 100 µl	250 µl & 500 µl
1k Da MWCO	7428-RC1K	7429-RC1K
2k Da MWCO	7428-RC2K	7429-RC2K
5k Da MWCO	7428-RC5K	7429-RC5K
10k Da MWCO	7428-RC10K	7429-RC10K
25k Da MWCO	7428-RC25K	7429-RC25K
50k Da MWCO	7428-RC50K	7429-RC50K

### Cellulose Acetate Membranes

100 Da MWCO	7425-CA100	7426-CA100
500 Da MWCO	7425-CA500	7426-CA500
1k Da MWCO	7425-CA1K	7426-CA1K
2k Da MWCO	7425-CA2K	7426-CA2K
5k Da MWCO	7425-CA5K	7426-CA5K
10k Da MWCO	7425-CA10K	7426-CA10K
25k Da MWCO	7425-CA25K	7426-CA25K
50k Da MWCO	7425-CA50K	7426-CA50K
100k Da MWCO	7425-CA100K	7426-CA100K
300k Da MWCO	7425-CA300K	7426-CA300K

### Polycarbonate Membranes

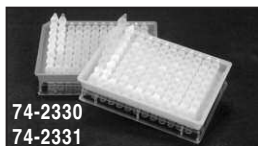
0.01 µm	7432-PC01	7433-PC01
0.05 µm	7432-PC05	7433-PC05
0.10 µm	7432-PC10	7433-PC10
0.60 µm	7432-PC60	7433-PC60



# Products

## 96-Well Equilibrium DIALYZER™ (U.S. Pat. 6,458,275)

The 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 96-Well Equilibrium DIALYZER™ and sample recovery is excellent. Wells are sealed with 8-cap strips or a plate seal mat with 96 inserts. 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 regenerated cellulose membranes with MWCO of 5,000 or 10,000 Daltons.



### APPLICATIONS

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

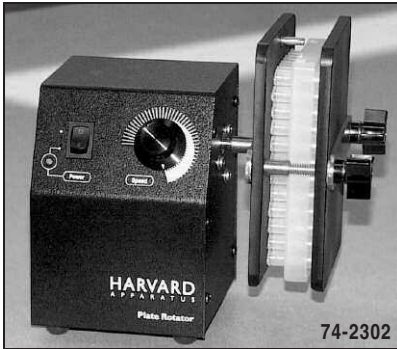
### ADVANTAGES

- 96-well format
- Individual membrane for each well
- Small sample volumes: 50 to 300µl
- Regenerated cellulose membranes
- Membranes are free of sulfur and other heavy metals
- High well-to-well reproducibility
- Excellent sample recovery (>95%)

Catalog No.	Description	Quantity
74-2330	96-Well Equilibrium DIALYZER™ Plate, Membrane MWCO 5K Daltons	1
74-2331	Equilibrium DIALYZER™ Plate, Membrane MWCO 10K Daltons	1
74-2323	8-Cap Strips	12
74-2322	Plate Seal Mat with Individual Well Inserts, Pierceable and Self-Sealable	2

# Products

## Plate Rotators



A Single or Dual Plate Rotator with variable rotation rates or a 8-Plate Rotator/Incubator is available for use with Harvard Apparatus' 96-Well Equilibrium DIALYZER™. The Rotator speeds up the equilibrium dialysis process by keeping the sample in constant motion ensuring higher reproducibility of results.

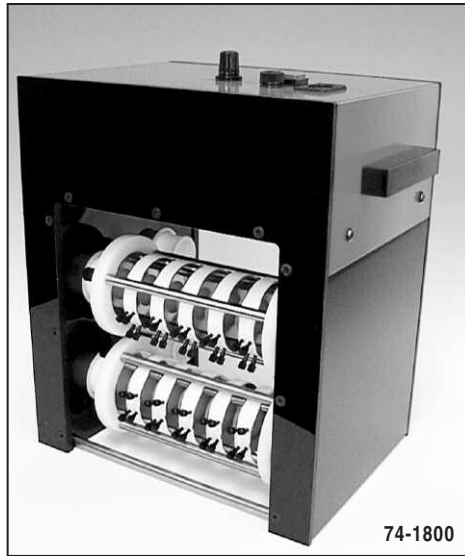
Catalog No.	Description	Quantity
74-2302	Single Plate Rotator	1
74-2334	Dual Plate Rotator	1
74-2335	8 Plate Rotator/Incubator	1



# Products

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## Multi-Equilibrium DIALYZER™

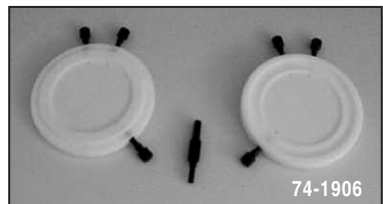


The Harvard Apparatus' Multi-Equilibrium DIALYZER™ provides highly standardized equilibrium dialysis conditions for up to 20 parallel assays. The instrument offers outstanding uniformity of:

- 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 (0.2, 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.



# Products

## Multi-Equilibrium DIALYZER™ (Continued)

### APPLICATIONS

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

### ADVANTAGES

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

Catalog No.	Description	Quantity
<b>Multi-Equilibrium DIALYZER™ Systems</b>		
74-1800	Complete Multi-Equilibrium DIALYZER™ System	
	- Ready-to-Use Teflon® Macro Dialysis Cells (1ml)	20
	- Variable Speed Drive Unit for 20 Cells	1
	- Stand	1
	- Carriers for 5 Teflon® Dialysis Cells	4
	- Macro Spacers	24
	- Dialysis Membranes MWCO 10K Daltons	200
<b>Membranes for Multi-Equilibrium DIALYZER™</b>		
74-2100	MWCO 5K Daltons	200
74-2102	MWCO 10K Daltons	200
<b>Multi-Equilibrium DIALYZER™ Individual Components</b>		
74-1913	Filling Clamp	1
74-1901	Emptying Stoppers	5
74-1914	Black Plugs	32
74-1907	Micro Teflon® Dialysis Cells (0.2 ml)	5
74-1906	Macro Teflon® Dialysis Cells (1 ml)	5
74-1904	Macro Teflon® Dialysis Cells (2 ml)	5
74-1905	Macro Teflon® Dialysis Cells (5 ml)	5

# Products

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## Graphpad Prism®

Graphpad Prism® combines nonlinear regression (curve fitting), basic biostatistics, and scientific graphing. Prism's unique design will help you efficiently analyze, graph, and organize your experimental data. Prism helps you in many ways:

Fit curves with nonlinear regression. For many labs, nonlinear regression is the most commonly used data analysis technique. No other program streamlines (and teaches) curve fitting like Prism.

Perform statistics. Prism makes it easy to perform basic statistical tests commonly used by laboratory researchers and clinicians. Prism does not take the place of heavy duty statistics programs. Prism offers a complete set of statistical analyses up to two-way ANOVA, including analysis of contingency tables and survival curves. Prism does not perform ANOVA higher than two-way, or multiple, logistic or proportional hazards regression.

Create scientific graphs. Prism makes a wide variety of 2D scientific graphics. Included are all the features that scientists need including automatic calculation of error bars, Greek letters, log axes, discontinuous axes and much more.

Organize your work. Prism's unique organization helps you stay organized and lets you carefully track how all your data are analyzed. Your data and files are linked into one organized folder so it is always easy to retrace your steps.

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