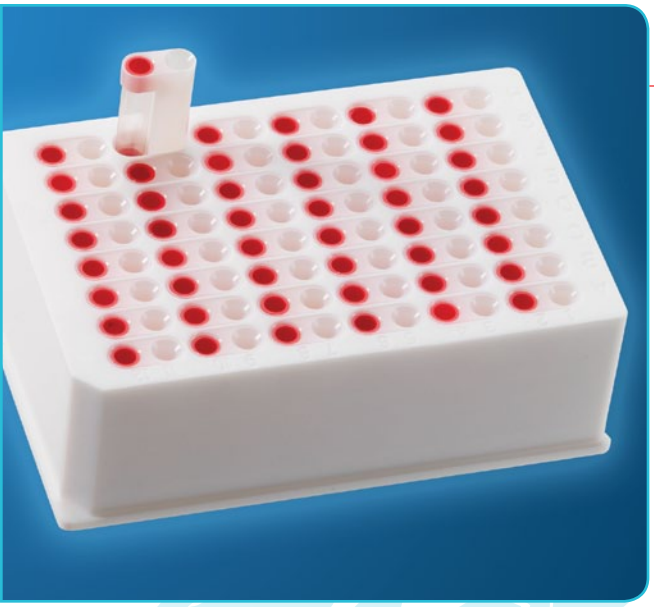
The Thermo Scientific RED Device Systems Brochure

plasma and tissue protein binding

The Original Thermo Scientific RED Device System • The Single-Use RED Device System • The Competition RED System

Thermo SCIENTIFIC



the RED technology

Determining the extent to which a molecule binds to plasma or tissue proteins is a critical phase of drug development because the amount of the bound drug influences compound dosing, efficacy, clearance rate and potential for drug interactions. This determination is enabled by equilibrium dialysis (Figure 1), an accepted and standard method for reliable estimation of the non-bound drug fraction in plasma. Although it is the preferred method, equilibrium dialysis has historically been labor-intensive, time-consuming, cost-prohibitive and difficult to automate.

Highlights:

- **Ease of use** – disposable tubes require no presoaking, assembly or specialized equipment
- **Short incubation time** – the high membrane surface-to-volume ratio enables equilibrium to be reached in as few as 100 minutes with vigorous agitation or in three to four hours with 200 rpm agitation
- **96-well format** – suitable for automated liquid handlers
- **Flexible** – can be used for any number of assays (1-48 assays/plate) without wasting the entire plate
- **Robust** – compartmentalized design eliminates potential for cross contamination or leakage
- **Reproducible and accurate** – validated for plasma-binding assays, producing results consistent with those reported in literature (Table 2)†
- **Validated** – each lot is functionally tested in a protein-binding assay for guaranteed performance

Applications:

- Plasma protein-binding assays
- Drug partition between plasma and whole blood†
- Determination of protein binding of liver microsomes to improve the correlation between *in vitro* and *in vivo* intrinsic clearance
- Drug binding competition between tissues vs. plasma proteins
- Pharmacokinetics studies
- Formulation of drug dosage for *in vivo* studies
- Drug-drug interaction studies
- Selection criteria during drug lead optimization
- Solubility studies
- Dissociation constant determination (Kd)
- Tissue-binding studies using tissue homogenate

† Review example protocols at thermoscientific.com/pierce

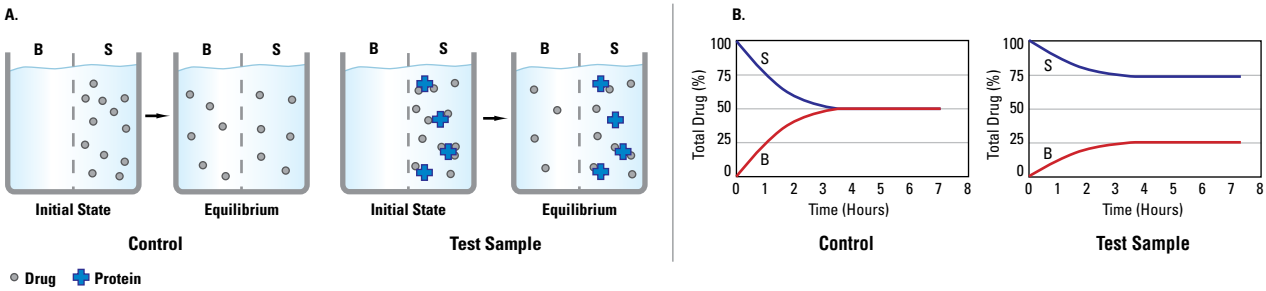
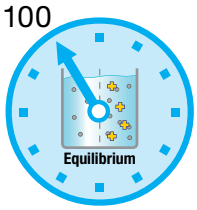


Figure 1. Panel A. Schematic of equilibrium dialysis for a control and protein-containing sample. Panel B. Ligand concentration over time in buffer chambers (B) and sample chambers (S). In a control sample, ligand is placed in one chamber and allowed to diffuse. When equilibrium is reached after four hours, ligand concentration is equal in each chamber. In the test sample example, the ligand and an interacting protein are placed in one chamber. When equilibrium is reached, 75% of the total ligand concentration remains in chamber S from protein binding.

The Thermo Scientific RED Device for Rapid Equilibrium Dialysis and Competition RED Device were developed in close association with the pharmaceutical industry to specifically address the labor, time, cost and automation concerns associated with traditional equilibrium devices, including accelerating lead optimization and reducing attrition rates (Table 3).

100-Minute Equilibration Procedure

The Thermo Scientific Single-Use RED Device System



To review the RED Device protocol for using dynamic agitation to reduce equilibration time during equilibration dialysis, visit thermoscientific.com/pierce. The protocol uses various compounds for plasma protein- and microsomal protein-binding studies. Protein-binding results are consistent with those reported in the literature (Table 1) and equilibration time was reduced from five hours to less than two hours (Figure 2).

Table 1. Plasma protein-binding results for seven compounds using the Thermo Scientific RED Device 100-minute protocol are equivalent to results achieved using classic devices found in the literature.

Compound	Human Plasma (% Bound)	SD	Literature
Warfarin	99.24	0.03	99
Taxol	96.16	0.36	95-98
Propranolol	91.81	0.19	80-92
Vinblastine	99.30	0.14	99
Verapamil	90.31	0.55	88-92
Atenolol	3.50	7.36	< 5
Antipyrine	0		0

1. A time course study using vortex agitation at 750rpm and 37°C was used to determine the free fraction at equilibration.

2. Most compounds were completed in fewer than 120 minutes.

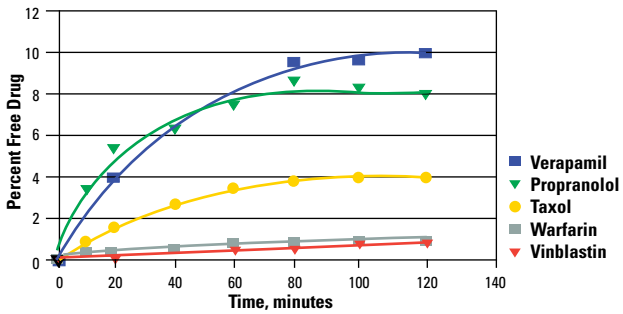


Figure 2. Equilibration time for these compounds (1µM of drug concentration in human plasma) was reduced from five hours to less than two hours using the dynamic agitation protocol.

The Original

The Original Thermo Scientific RED Device System

The original RED Device System consists of disposable tube inserts with a large surface area to achieve equilibrium faster and a uniquely designed 96-well PTFE base plate that provides compatibility with automated liquid handling systems. The plate includes 48 inserts. Each insert is made of two side-by-side chambers separated by a vertical cylinder of 8K MWCO dialysis membrane validated for minimal nonspecific binding. The RED Device has been extensively validated for plasma-binding assays, producing results consistent with those reported in the literature (Table 2).

- **Versatile** – the original RED Device base plate is made of chemically inert high-grade PTFE, eliminating nonspecific binding and risk of contamination

Table 2. Comparison of results obtained using the Thermo Scientific RED Device with values reported in the literature.

Compound	% Bound	
	RED Device	Literature Value
Ranitidine[1]	17	10-19
Warfarin[3]	99	99
Naproxen[1]	99	99
Taxol	96.16	95-98
Propranolol[2]	91.81	80-92
Vinblastine	99.30	99
Verapamil	90.31	88-92
Atenolol	3.50	< 5
Antipyrine	0	0

1. Jusko, W.J. and Gretch, M. (1976). Plasma and tissue protein binding of drugs in pharmacokinetics. *Drug Metab. Rev.*, **5**(1), 42-139.
2. Colangelo, P.M., *et al.* (1992). Age and propranolol stereoselective disposition in humans. *Clin. Pharmacol.* Ther., **51**, 489-94.
3. Chan, E., *et al.* (1994). Disposition of warfarin enantiomers and metabolites in patients during multiple dosing with rac-warfarin. *Brit. J. Clin. Pharmacol.*, **36**, 563-569.

Table 3. The Thermo Scientific RED System offers significant improvements in the ease of use, time requirements, versatility and product reliability compared to other commercially available devices.

Device	Time to reach Equilibrium	Leakage	Disposable	Labor Intensity	Automation Accessible	Vol. Shift
Multi-Equilibrium DIALYZER (Dianorm, Harvard, Spectrum)	3-4h	Some	No	++++	No	Minimum
96-Well Equilibrium DIALYZER †† (Harvard Apparatus)	16h	Some	Yes	+++	Possible	Yes
96-well Micro-Equilibrium Dialysis (HTDialysis, LLC)	6h	Some	No	+++	Possible	Yes
RED Device (Thermo Fisher Scientific)	4~6h (2h)	None	Yes	+	Yes	None

Comparison done by Millennium Pharmaceuticals, Inc., 2005. ††Product has been modified since Millennium Study.

The Single Use

The Thermo Scientific Single-Use RED Device System

The Thermo Scientific Single-Use RED System consists of a disposable plate made of high-density polypropylene that is preloaded with 48 equilibrium dialysis inserts. Each insert is made of two side-by-side chambers separated by a vertical cylinder of 8K, 12K, or 25K MWCO dialysis membrane validated for minimal nonspecific binding. This pre-loaded disposable device is automation-friendly, providing convenience for scientists conducting protein-binding studies. The inserts are ready to use and do not require any pre-conditioning to the membrane. The single-use format is especially convenient for labs using radioactive materials because the plate can be disposed of easily to avoid contamination and cleaning.

The Single-Use RED Device has been validated in human plasma protein-binding assays with high-, medium- and low-protein binding compounds with results equivalent to those obtained using the original RED Device base plate made from PTFE material and to those results reported in the literature (Table 5).[1]

Table 4. Recommended membrane for various drug candidates.

MWCO	Small Molecules	siRNA	Peptides
8K	x		
12K	x		
25K	x	x	x

Table 5. Performance of pre-loaded Thermo Scientific Single-Use RED Base Plates using high-, medium- and low-protein-binding compounds tested at 1 µM on human plasma.

Compound	Human Plasma (% Bound)	
	RED Device Plate	*Other Device[1-4]
Warfarin	99.24	99
Taxol	96.16	95-98
Propranolol	91.81	80-92
Vinblastine	99.30	99
Verapamil	90.31	88-92
Atenolol	3.50	< 5
Antipyrine	0	0

1. Brunton, L., *et al.* (2005). Goodman and Gilman's Pharmacological Basis of Therapeutics. McGraw Hill Professional Publishing: New York.
2. Clausen, J. and Bickel, M. (1993). Prediction of drug distribution in distribution dialysis and *in vivo* from binding to tissues and blood. *J. Pharm. Sci.* **82**, 345-349.
3. Sonnichsen, D. and Relling, M. (1994). Clinical pharmacokinetics of paclitaxel. *Clin. Pharmacokinet.* **27**, 256-269.
4. Steele, W., *et al.* (1983). The protein binding of vinblastine in the serum of normal subjects and patients with Hodgkin's disease. *Eur. J. Clin. Pharmacol.* **24**: 683-687.

Ordering Information

Product #	Description	Pkg. Size
90006	**Single-Use RED Plate with Inserts** Each plate contains 48 inserts.	1 plate
90007	**Single-Use RED Plate with Inserts** Each plate contains 48 inserts.	5 plates
90004	**Single-Use RED Base Plate (Empty)**	2/pkg.
90005	**Single-Use RED Base Plate (Empty)**	10/pkg.
89809	**RED Device Inserts**	50/pkg.
89810	**RED Device Inserts**	250/pkg.
89811	**Base Plate – Made of PTFE Material**	1/pkg.
89812	**RED Device Insert Removal Tool**	1 ea.
15036	**Sealing Tape for 96-Well Plates**	100/pkg.
99006	**RED Device Single-Use Plate with Inserts, 8K MWCO** Formulation: High-density polypropylene plate with RED Device Inserts *Sufficient For: 10 × 48 experiments*	10-plate set
90112	**RED Device Single-Use Plate with Inserts, 12K MWCO** Formulation: High-density polypropylene plate with RED Device Inserts *Sufficient for: 1 × 48 experiments*	1-plate set
91012	**RED Device Single-Use Plate with Inserts, 12K MWCO** Formulation: High-density polypropylene plate with RED Device Inserts *Sufficient for: 10 × 48 experiments*	10-plate set
90125	**RED Device Single-Use Plate with Inserts, 25K MWCO** Formulation: High-density polypropylene plate with RED Device Inserts *Sufficient for: 1 × 48 experiments*	1-plate set

The Competition Binding System

The Thermo Scientific Competition RED System

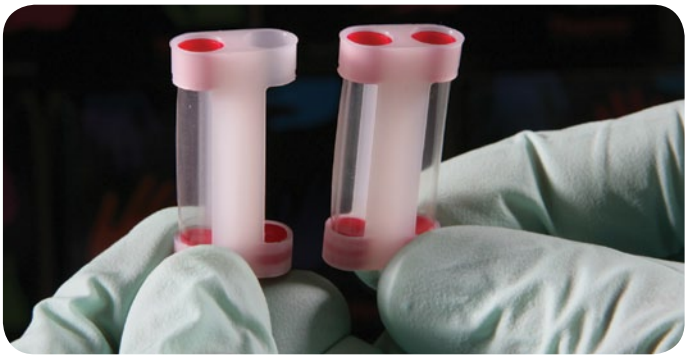


Figure 4. The different wells allow a choice of the size best suited for the specific experimental design. The plate and lid are made of high-grade reusable PTFE. The lid snaps onto the base plate and holds and suspends the disposable dialysis inserts in the wells to expose samples to a common dialysis matrix. RED Device Insert pictured on left and Competition RED Device Insert pictured on right.

Highlights:

- **Unique concept** – determine *in vitro* tissue protein binding from two to 15 different tissue samples simultaneously in about four hours
- **Reproducible and accurate** – perform controlled experiments with multiple tissues to rank your compounds before doing *in vivo* studies
- **Easy and ready to use** – disposable tubes require no presoaking step, assembly or specialized equipment
- **Robust** – compartmentalized design eliminates potential for crosstalk or leakage
- **Validated quality** – base plate is composed of chemically inert, high-grade PTFE, eliminating nonspecific binding

Applications:

- Preliminary drug candidate screening in ADME-Tox studies – *in vitro* screening of drug partitioning between plasma and multiple tissues before *in vivo* studies
- Hit-to-lead selection of new chemical entities (NCE) for preclinical studies
- Competitive binding and dissociation constant determination for small molecules versus multiple targets
- Determining formulation of drug dosage for *in vivo* studies

The Competition RED System consists of disposable dialysis tube inserts and a reusable PTFE base plate. The unique base plate design allows placement of 3 to 15 dialysis chambers into a common well enabling researchers to perform several experiments simultaneously (Figures 5 and 6). The Competition RED Device has a standard 96-well plate footprint with 9 x 9mm well spacing. Additionally, the small volume and large dialysis surface area of the tube inserts allows rapid dialysis, achieving equilibrium in about four hours with high levels of reproducibility and accuracy.

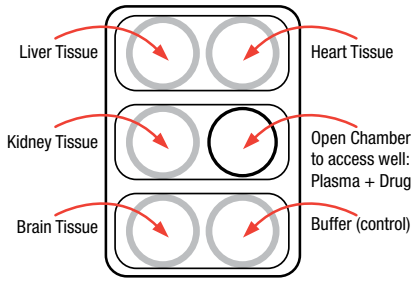


Figure 5. Example device setup for monitoring drug partitioning. Each 10-pack of Competition RED Inserts contains eight dual-chamber inserts and two single-chamber inserts. The open chamber in the single-chamber inserts enables direct access to the sample in the base plate well without disassembling the device.

Design of the multiplex dialysis system mimics the configuration of unbound drug in plasma diffusing into different discrete tissues while maintaining a common fraction of unbound drug.

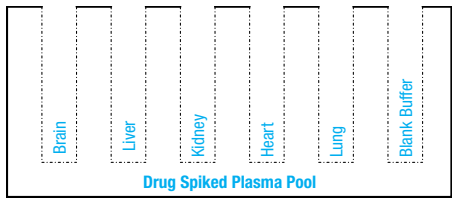


Figure 6. Design of the multiple membrane chambers immersing in the plasma pool. A blank buffer that can provide information of apparent systemic-free drug content at equilibration stage.

Ordering Information

Product #	Description	Pkg. Size
90087	Competition RED Inserts	10/pkg.
90088	Competition RED Inserts	50/pkg.
90085	Competition RED Base Plate	1 unit

Thermo Scientific Pierce Protein Precipitation Plates

Our 96-well filtration plates are specifically designed for protein precipitation applications.

Highlights:

- Prepare small molecule samples for HPLC or LC-MS in 10-30 minutes
- Work seamlessly with the RED Device and Competition RED Device
- No acetonitrile leakage for up to four hours
- Leach-free graded hydrophobic frit prevents blockage
- Easily collect filtrates by vacuum, positive pressure or centrifugation
- Compatible with most organic solvents

Ordering Information

Product #	Description	Pkg. Size
90036	Protein Precipitation Plates	2 plates
90037	Protein Precipitation Plates	10 plates

Tech Tip!

Dilution Method

Use of dilution method to differentiate high protein-bound compounds or to examine free drug fractions in rare plasma or tissue samples.

A small difference in high protein-bound tissue compounds often causes large differences in free drug molecules. However, it is difficult to detect minute differences without experimental errors and many clinical tissue samples are available only in limited quantities. A dilution approach is advantageous because it allows for obtaining the free drug information while using minimal sample quantities, the dilution method can also be used to study precious and rare clinical samples to obtain undiluted fraction values.

Plasma or tissue samples can be diluted as much as 20-fold. The results below were achieved using a compound spiked five-fold with diluted plasma, followed by performing a standard protein binding procedure. For most samples a phosphate-buffered saline (PBS) with pH7.4 was used. For highly lipophilic compounds, five-fold diluted plasma water was used. The diluted free drug fractions ($f_{u'}$) can be converted to undiluted free drug fractions (f_u) using Formula (A). For validation, the data are calculated from concentrations and area ratios. Small differences in undiluted f_u can be amplified to give larger differences in diluted $f_{u'}$ (Figure 6). This dilution method demonstrates a convenient method for identifying the difference in highly bound compounds.

Formula A.

$$f_u = \frac{a * f_{u'}}{1 - f_{u'} * (1-a)}$$

f_u: **Undiluted free drug fraction**
$f_{u'}$: **Diluted free drug fraction**
a: **Dilution factor** (a=0.1 for 10-fold dilution; a=0.2 for a 5-fold dilution)

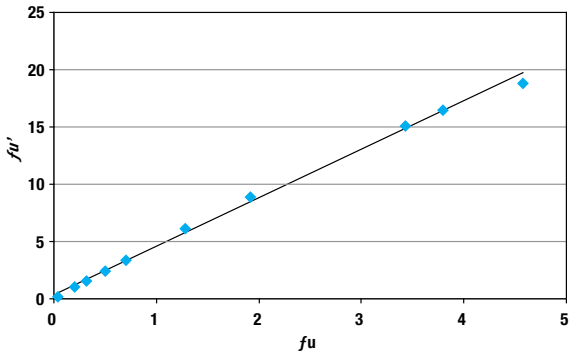


Figure 6. Plot of undiluted free drug fraction (f_u) vs. diluted free drug fraction ($f_{u'}$).

Table 6. Dilution factor for five-fold dilution.

f_u – undiluted	0.04	0.20	0.32	0.50	0.70	1.28	1.92	3.43	3.80	4.58
$f_{u'}$ – diluted	0.19	1.00	1.56	2.45	3.40	6.09	8.90	15.09	16.49	19.31

References:

J. Pharmaceutical Sciences, Vol. 89, #8, August 2000
J. Med.Chem. (2007), **50**, 4606-4615

Tech Tip!

Tissue Distribution

Unbound drug plays a key role in tissue distribution.

Multiple tissue-to-plasma binding ratios (e.g., Kp) can be obtained from a single experiment using the Thermo Scientific Competition RED Device with multiple tissues in a pool of compound spiked plasma. The Competition RED design mimics the configuration of a drug circulating in the body as tissues compete for the free drug being dynamically released from the drug-bound plasma. Data obtained from the *in vitro* Competition RED Device can be directly compared with data from *in vivo* studies to screen a list of potential drug candidates and select the most desirable distribution profile for an *in vivo* study. For example, studies using propranolol (Figure 7) and Taxol® (Figure 8) were conducted to demonstrate the tissue distribution correlations between *in vitro* and *in vivo* studies.

The following charts compare results of the *in vitro* and *in vivo* studies:

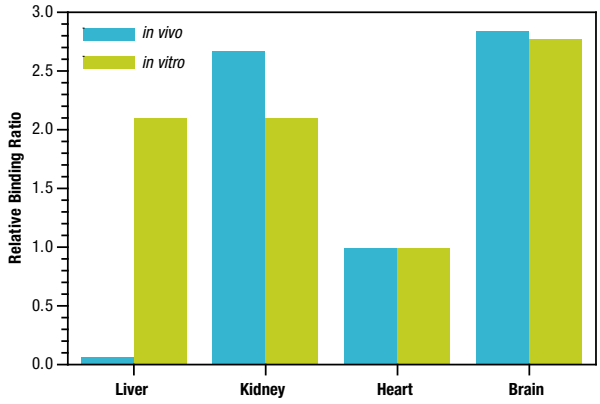


Figure 7. Comparison of *in vitro* and *in vivo* tissue and plasma-binding ratios of propranolol in rat. Low propanolol presence in liver tissue *in vivo* is mainly due to high hepatic metabolic activities.

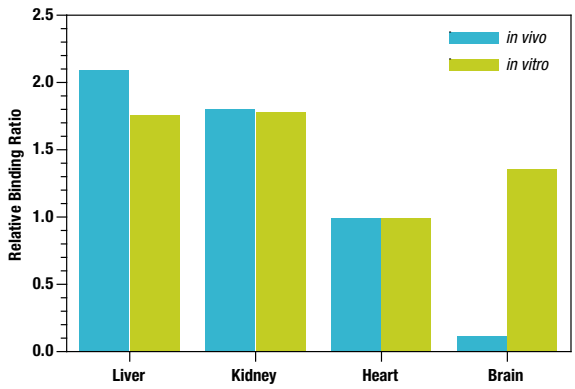
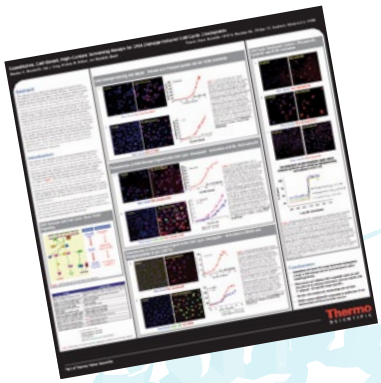


Figure 8. Comparison of *in-vitro* and *in-vivo* tissue/plasma binding ratios of Taxol in rat. Low Taxol presence in brain tissue *in vivo* indicates the blood-brain barrier inhibits movement of Taxol to the brain.



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