Equilibrium Dialysis - RED Devices

Summary:

Rapid Equilibrium Dialysis (RED) Device

A transforming technology for plasma protein-binding assays.

The Pierce RED Device for Rapid Equilibrium Dialysis provides the easiest, fastest and most reliable system for performing plasma protein-binding assays, a critical step in drug development.

The Device for rapid equilibrium dialysis (RED) was developed in association with the pharmaceutical industry to provide the easiest, fastest and most reliable system for performing plasma protein-binding assays. The RED Device is an apparatus for performing equilibrium dialysis experiments in a high throughput, automation-compatible format. The RED device consists of disposable inserts and a base plate formatted to a standard microplate footprint. The RED Device has been extensively validated for plasma-binding assays and produces results consistent with those reported in the literature. The RED Device offers significant improvements in automation, time requirements, versatility and product reliability compared to other commercially available equilibrium dialysis systems.

Highlights:

- **Easy and ready to use** – disposable tubes require no presoaking, assembly or specialized equipment
- **Designed for speed** – the high surface-to-volume ratio of the insert design enables equilibrium to be reached in as few as 100 minutes with vigorous agitation or in three to four hours with 200rpm agitation
- **Automation-compatible** – designed on a standard 96-well plate template suitable for automated liquid handlers
- **Flexible and scalable** – perform any number of assays (1 to 48 assays per plate) without wasting the entire plate
- **Robust** – compartmentalized design eliminates potential for cross talk or leakage
- **Reproducible and accurate** – validated for plasma binding assays, producing results consistent with those reported in literature
- **Quality-tested** – each lot of inserts is functionally tested in a protein-binding assay for guaranteed performance

Features of the RED Device Inserts:

Each single-use, disposable insert is made of two side-by-side chambers separated by a vertical cylinder of dialysis membrane (in 8K or 12K MWCO) validated for minimal nonspecific binding. This format requires no extensive assembly steps or specialized equipment, and each chamber or well is easily accessible from the top of the insert after insertion in the base plate. Additionally, the high surface-to-volume ratio of the membrane compartment allows rapid dialysis, where equilibrium can be reached in 4 hours with high levels of reproducibility and accuracy. In many cases, experiments can be completed in less than 100 minutes.

Insert Features:

- Disposable: require no presoaking, assembly, or specialized equipment
- Short incubation time: large dialysis surface area accelerates equilibrium
- 8K MWCO membrane: ideal molecular-weight cutoff for protein-drug binding studies
- 12K MWCO membrane also available for larger drug molecules
- Membrane composition: regenerated cellulose with low glycerol content as a humectant

Features of the RED Device Base Plates:

RED Device Inserts are designed to be used with either the reusable PTFE or disposable high-density polypropylene base plates. Each RED Device Base Plate holds up to 48 RED Device Inserts and has a standard 96-well plate footprint with 9mm x 9mm well spacing to provide compatibility with automated liquid handling systems. In addition, the disposable RED Device Base Plates are available pre-loaded, providing operation convenience for scientists conducting protein-binding applications. No pre-conditioning of the membrane inserts is needed. When using radioactive materials, this single-use plate is easily disposed of to avoid contamination and cleaning. RED Device Inserts and Base Plates are also available

separately.

Base Plate Features:

- Microplate footprint: compatible with automated systems for 96-well plates
- Compartmentalized: eliminates potential for crosstalk or leakage
- PTFE construction: eliminates nonspecific binding and risk of contamination
- Accepts 1 to 48 inserts: run exactly the number of assays needed without waste

Applications:

- Determination of free vs. drug bound to plasma proteins
- Pharmacokinetics studies
- Formulation of drug dosage for *in vivo* studies
- Drug-drug interaction studies
- Selection criteria during drug lead optimization
- Drug partition between plasma and whole blood
- Solubility study
- Dissociation constant determination (K_d)
- Tissue-binding study using tissue homogenate

Ordering Information

Product #	Description	Pkg. Size	Instructions	MSDS	CofA	Price
90006	**RED Device Single-Use Plate with Inserts, 8K MWCO** High-density polypropylene plate with RED Device Inserts *Sufficient For: 1 × 48 experiments*	1-plate set				
90007	*Sufficient For: 5 × 48 experiments*	5-plate set				
99006	*Sufficient For: 10 × 48 experiments*	10-plate set				
89809	RED Device **Inserts**, 8K MWCO Plastic framed cellulose membrane tube. *Sufficient For: 50 equilibrium dialysis experiments with a RED Base Plate (sold separately)*	50 inserts				
89810	*Sufficient For: 250 equilibrium dialysis experiments with a RED Base Plate (sold separately)*	250 inserts				
90112	**RED Device Single-Use Plate with Inserts, 12K MWCO** High-density polypropylene plate with RED Device Inserts *Sufficient For: 1 × 48 experiments*	1-plate set				
91012	*Sufficient For: 10 × 48 experiments*	10-plate set				
89811	RED Device Reusable **Base Plate** High-grade PTFE. *Sufficient For: Unlimited (reusable) 48-well experiments with RED Inserts (sold separately)*	1 plate				
90004	RED Device Single-Use Base Plates High-density polypropylene. *Sufficient For: 2 × 48 experiments (empty plates; require RED Inserts; sold separately)*	2 plates				
90005	*Sufficient For: 10 × 48 experiments (empty plates; require RED Inserts; sold separately)*	10 plates				
89812	RED Device Insert **Removal Tool** Stainless steel. *Sufficient For: Removing columns of RED Inserts from RED Base Plates (reusable)*	1 tool				

More: product details | References | FAQ

Product Details:

Determining the extent to which a molecule binds to plasma proteins is a critical phase of pharmaceutical development because it influences compound dosing, drug efficacy, clearance rate and potential for drug interactions. This determination is enabled by equilibrium dialysis, an accepted standard method for reliable estimation of the nonbound drug fraction in plasma. Although it is the preferred method, equilibrium dialysis is generally labor-intensive, time-consuming, cost-prohibitive and difficult to automate. The RED Device for rapid equilibrium dialysis was developed in close association with the pharmaceutical industry to specifically address these issues, accelerating lead optimization and reducing the attrition rate. In addition to plasma protein binding, the device is used for determining drug partition between red blood cell and plasma, protein binding of liver microsomes to improve the correlation between in vitro and in vivo intrinsic clearance, the competition between tissue protein binding against plasma proteins and dissociation constant determination (Kd).

The RED Device has been extensively validated for plasma-binding assays producing results consistent with those reported in the literature. For example, when used to measure warfarin binding to plasma (human and rat) proteins at two concentrations of 1µM and 10µM, the RED Device produced results with minimal intra-experimental variability. The Rapid Equilibrium Dialysis (RED) Device offers significant improvements in automation, time requirements, versatility and product reliability compared to other commercially available systems.

Use the RED Device for increased productivity. Each RED Device Base Plate sits in a 96-well plate footprint withe 9 x 9mm well spacing making it compatible with automated liquid handling systems. Single-Use RED Base Plates are available with inserts preloaded mimimizing setup time.

The RED Device reproduces results found in the literature. Performance of pre-loaded Single-Use RED Device Base Plates (Part No. 90006) using high, medium and low protein-binding compounds tested at 1µM on human plasma

	Human plasma (% bound)	
Compound	**RED plate**	**Other Devices †**
	99.24	99
Warfarin	96.16	95 to 98
Taxol	91.81	80 to 92
Propanolol	99.30	99
Vinblastine	90.31	88 to 92
Verapamil	3.50	< 5
Atenolol	0	0

† Values reported in the literature (Cited References 2-6).

The RED Device for comparison of plasma and microsome samples. Comparison of protein bindings between human plasma and human microsome at 1µM concentration as determined using the Single-Use RED Device. Microsomal protein concentration of 1.0mg/mL was used in the study.

Compound	**Human Plasma, % free**	**Human Microsome, % free**
Warfarin	< 1	81
Taxol	4	20
Propanolol	8	44
Vinblastine	0.7	4
Verapamil	10	27
Methotrexate	50	70
Simvastatin	7	23
Atenolol	97	100
Antipyrine	100	100

The RED Device had many advantages to other methods. The RED Device System offers significant improvements in the ease of use, time requirements, versatility and product reliability compared to other methods. Equilibrium can be reached in as little as 100 minutes with mild agitation or 3-4 hours with no agitation.

Device	**Time to reach Equilibrium**	**Disposable**	**Labor Intensity**	**Automation Accessible**	**Vol. Shift**

RED (Rapid Equilibrium Dialysis) Device	4 hours	Yes	+	Yes	None
96 - well Micro Equilibrium Dialysis block (HTDDialysis, LLC)	6 hours	No	+++	Possible	Yes
24-Multiwell Dialysis (BD Biosciences)	24 hours	Yes	++	Possible	Not measured

Data are from the following authors: Shelley Li[1], Bob Xiong[2], Tainang Huang[2], Lily Li[2], John Donovan[3], Frank Lee[1], Shaoxia Yu1, Gerald Miwa[1], Hua Yang[1] (Institutions: [1]DMPK/Drug Safety & Disposition, and [3]Process Technology, Millennium Pharmaceuticals, Inc. 40 Landsdowne Street, Cambridge, MA 02139 USA; and [2]Linden Bioscience, 35A Cabot Road, Woburn, MA 01801, USA).

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References:

1. Waters, N.J., *et al.* (2008). Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. *J Pharm Sci* **97(10)**: 4586-95.
2. Brouwer, E.J., *et al.* (2000). Measurement of fraction unbound paclitaxel in human plasma. *Drug Metab Disposition* **28(10)**: 1141-5.
3. Brunton, L., *et al.* Goodman and Gilman's Pharmacological Basis of Therapeutics. McGraw Hill Publishing: New York, 2005.
4. Clausen, J. and Bickel, M. (1993). Prediction of drug distribution in distribution dialysis and *in vivo* from binding to tissue and blood. *J Pharm Sci* **82**: 345-9.
5. Sonnichsen, D. and Relling, M. (1994). Clinical pharmacokinetics of paclitaxel. *Clin Pharmacokinet* **27**: 256-69.
6. Steele, W., *et al.* (1983). The protein binding of vinblastrine in the serum of normal subjects and patients with Hodgkin's disease. *Eur J Clin Pharmacol* **24**: 683-7.

Competition Rapid Equilibrium Dialysis (RED) Devices

A transforming technology for plasma protein-binding assays.

The Pierce Competition RED Device Inserts and Base Plates comprise a specially designed system for evaluating competitive binding interactions using rapid equilibrium dialysis (RED).

The Competition RED Device was developed in association with pharmaceutical laboratories to more accurately model *in vivo* drug interactions by enabling equilibrium dialysis experiments to be performed in a multiplexed format involving competition for binding among different tissues. The Competition RED Device System consists of disposable dialysis tube inserts and a reusable Base Plate made of high-grade PTFE. The Base Plate is much like our regular RED Device Plate except that it is divided into different size chambers (wells) for positioning 2-8 RED Device Inserts per well. The format enables competitive dialysis experiments involving 2-15 separate tissue or protein fluid samples. Each Competition RED Insert contains either one or two separate dialysis chambers (each package includes a selection of both types). The Competition RED Device requires no extensive assembly or specialized equipment, and each chamber/well is easily accessible from the top of the device.

Highlights:

- **Easy to use** – disposable tubes require no presoaking step, assembly, or specialized equipment
- **Short incubation time** – design provides high surface-to-volume ratio of membrane to sample, enabling equilibrium to be reached within 2-4 hours
- **Flexible format** – base plate contains several different chamber sizes, enabling small molecule partitioning studies involving 2-16 tissue or protein samples without waste
- **Robust** – compartmentalized design eliminates potential for cross talk or leakage
- **Reproducible and accurate** – perform controlled experiments with multiple tissues to obtain screening results that have high predictability for *in vivo* studies with animal models
- **Validated quality** – base plate is composed of chemically inert high-grade PTFE, eliminating non-specific binding and risk of contamination; each lot is functionally tested in a protein binding assay for guaranteed performance

Applications:

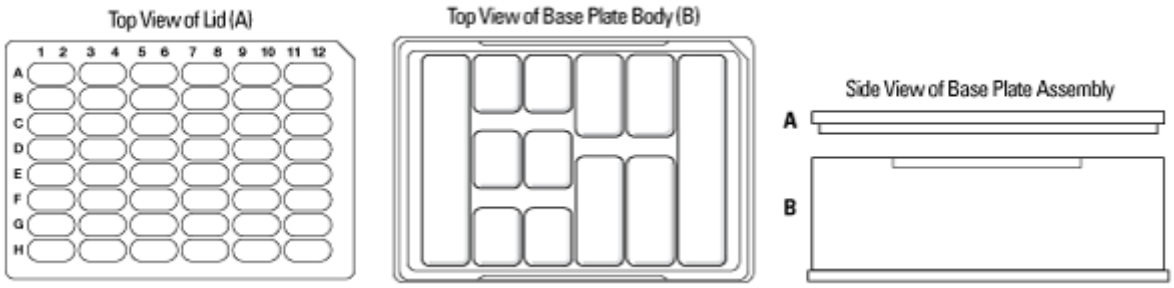
- ADME-Tox studies: *in vitro* screening of drug partitioning between plasma and multiple tissues before *in vivo* studies with animal models
- Aids in determining formulation of drug dosage for *in vivo* studies
- Drug-drug interaction studies
- Competitive binding and dissociation constant determination for small molecules versus multiple targets

Ordering Information

Product #	Description	Pkg. Size	Instructions	MSDS	CofA	Price
90085	**Competition RED Base Plate** Contains base plate body and lid	1 unit				
90087	**Competition RED Inserts** Contains 8 dual-membrane inserts and 2 single-membrane inserts	10 / pack				
90088	Contains 40 dual-membrane inserts and 10 single-membrane inserts	50 / pack				

More: product details | FAQ

Product Details:

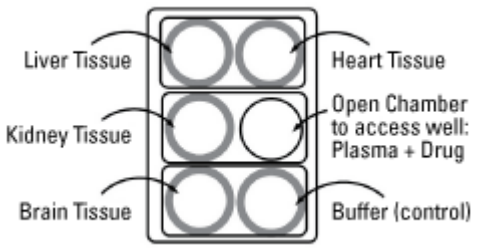


Format of the Competition RED Device Base Plate. The Base Plate body (center) has different well sizes for holding 4, 6, 8 or 16 dialysis chambers (2, 3, 4 or 8 inserts, respectively). The different wells allow a choice of the size best suited for the specific experimental design. The lid (left) snaps onto the Base Plate and holds and suspends the dialysis chambers in the wells.The device requires no extensive assembly steps or specialized equipment, and each chamber/well is easily accessible from the top of the device. The base plate has a standard 96-well plate footprint with 9 x 9 mm well spacing. The Competition RED Device Inserts (pictured above) are offered with the molecular weight cutoff (MWCO) of 12,000Da.

Sample volume required for each Base Plate well size. Add 0.5mL to each insert slot that is not filled.

Base Plate Well	Number of Inserts	Total Volume
4-compartment	2 inserts	1.5mL
6-compartment	3 inserts	2.5mL
8-compartment	4 inserts	3.0mL
16-compartment	8 inserts	5.0mL

Determining the extent to which a molecule partitions between plasma and specific tissues in the body is a critical phase of drug development because it determines compound dosing, efficacy, clearance rate, and the potential for drug interactions or tissue damage. Although final assessment of these variables invariably requires animal dosing and analysis, *in vitro* screening systems that are rapid and have good predictability are highly desirable for ADME-Tox studies. Equilibrium dialysis is among the most successful of *in vitro* screening method for evaluating drug binding and dosage requirements. Equilibrium dialysis is used to estimate the non-bound drug fraction in plasma or drug partitioning between red blood cell and plasma, plasma and liver microsomes, or plasma and tissue homogenates. However, many tissue-partitioning studies indicate that certain drugs show significant binding to tissues tested individually but have considerably different binding preferences when placed in competition between multiple tissues. The Competition RED Device extends the power of equilibrium dialysis by facilitating analysis of simultaneous drug interactions and partitioning among multiple tissues.



Example device setup and experiment. This setup uses a 6-compartment well of the base plate, two dual-chamber inserts (top and bottom) and one single-chamber insert (middle position). 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. This example experiment monitors drug partitioning among buffer, plasma, heart, liver, kidney and brain tissue extracts. The buffer chamber is for determining the amount of unbound drug.

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Rapid Equilibrium Dialysis Device FAQs

What is the membrane material in the RED Device Inserts?
Regenerated cellulose with a low glycerol content as a humectant.

What is the pore size or molecular weight cut-off (MWCO) for the membrane?

The nominal MWCO is 6,000-8,000 Daltons as specified by the manufacturer.

Is there any preprocessing, such as rinsing the RED Device Inserts, which is required before they can be used?
No. The inserts can be used directly out of the package, no presoaking or rinsing of the membrane is needed.

Are the units sterile or endotoxin free?
The units do not undergo a sterilization procedure nor are they tested for endotoxin content.

How long does it take to reach equilibrium dialysis?
In most cases 4 hours is sufficient to reach equilibrium. This is due to the high surface area to volume ratio for the membrane (7.4:1).

Why is the volume different between the membrane chamber and the buffer chamber? Will it affect the results?
The difference in liquid volume is to maintain the same level (height) between the two chambers. The equilibrium constant depends only on the concentration of the ligand, not the volume.

Is there a volume shift in either sample?
Volume shift in the plasma is unusual due to how quickly equilibrium can be reached with the RED Device. If much longer incubation times are used, there can be an increase in the volume of the plasma. However, the ligand will still reach equilibrium, having free ligand at the same concentration on both sides of the membrane.

Is there any non-specific binding of compounds to the device?
The container for the device is PTFE and the insert is made of high density polyethylene (HDPE) and are highly hydrophobic. The regenerated cellulose membrane is a standard material for commercial dialysis devices. A recovery study shows a consistency of 85% recovery of high and low protein binding compounds. This result is indicative of minimal non-specific binding.

What samples are typically tested for binding of ligands with the RED Device?
Plasma samples from human, mouse and rat. For toxicology studies, monkey is also typically tested. Typically pooled plasma samples purchased from commercial vendors are used, although researchers could test the differences in plasma from various physiological states using the RED Devices.

What is the minimum volume of plasma that I can use?
The lowest validated volume for the assay is 200µl of plasma. We are in the process of evaluating 100µl plasma samples.

Can the RED Devices be reused?
No, these are designed for a single use, as plasma is sticky and cannot be removed from the unit. The PTFE plate is designed for multiple uses.

If we use radioactive compounds in our study, will the PTFE plate be contaminated, preventing reuse?
No. After use, rinse the PTFE plate in 20% ethanol in water and rinse with ultrapure water at least two times. No radioactivity will remain after these rinses.

If my compound has low solubility in aqueous buffer, can I use DMSO and if so, at what concentration?

A final concentration of 1% DMSO is acceptable and will not affect the study. Have we tested DMF and other solvents? No, DMF has not been tested. However, it is expected that DMF should be similar to DMSO in terms of use.

How do I mix the samples to speed reaching equilibrium?
Once samples are loaded into the PTFE plate it should be placed on a shaking device. With an orbital shaker 100 rpm works well. For an up and down shaker, 20 rpm is sufficient.

If I see vapor condensing on the clear sealing film, will the loss of water vapor affect the result?
No. It will not affect the equilibrium.

Can I heat the PTFE block on a hot plate to maintain the temperature?
Yes, as most assays are performed at physiological temperature (37.5°C).

Why can I only process 48 samples on a 96-well plate?
Each sample requires two chambers for equilibrium dialysis to take place (96/2 = 48).

Related products - alternative dialysis devices for 0.3 to 10ml, that do not require syringes nor floating boys:

* Dialysis tool- - selection guide
* Accessory reagents: Protein Precipitation Plates Sealing Tape for Microplates
Phosphate Buffered Saline LC/MS Grade Acetonitrile Trifluoroacetic Acid
* Products HighLights Overview

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