Pierce™ RED Device Single-Use Plate with Inserts

Catalog Numbers 90006, 90007, 99006, 90112, 91012

Doc. Part No. 2162034 Pub. No. MAN0011619 Rev. C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from thermofisher.com/support.

Product description

The Pierce™ Rapid Equilibrium Dialysis (RED) Device Single-Use Plate with Inserts is composed of disposable high-density polypropylene and is preloaded with 48 equilibrium dialysis membrane inserts. Each insert includes 2 side-by-side chambers separated by an O-ring-sealed vertical cylinder of dialysis membrane with varying molecular-weight cutoffs (MWCO). This preloaded disposable device is automation-friendly, providing operation convenience for scientists conducting protein binding applications. No preconditioning of the membrane inserts is needed. When using radioactive materials, this single-use plate is easily disposed of to avoid contamination and cleaning. RED Inserts and Base Plates are also available separately (see “Related products” on page 3).

Equilibrium dialysis is an accurate and reliable method for determining protein binding affinities to chemical or biological substances of low molecular weight. The Pierce™ RED Device Single-Use Plate is specifically designed and extensively validated for plasma serum binding assays and produces results consistent with those reported in the literature (see “Additional information” on page 3). 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, and the competition between tissue protein binding against plasma proteins. The Pierce™ RED Device Single-Use Plate is validated for minimal nonspecific binding.

The design of the Pierce™ RED Device Single-Use Plate provides many advantages. This format 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 mm x 9 mm well spacing. Additionally, the high membrane surface-to-volume ratio enables rapid dialysis, where equilibrium can be reached in 4 hours with high levels of reproducibility and accuracy.

Go to our website for more detailed information about equilibrium dialysis and a video of the RED Device in action: www.thermofisher.com/us/en/home/life-science/protein-biology/protein-purification-isolation/protein-dialysis-desalting-concentration/dialysis-products/plasma-protein-binding-equilibrium-dialysis

Contents and storage

Item	Membrane Molecular Weight Cut-Off	Cat. No.	Amount	Storage
Pierce™ RED Device Single-Use Plate with Inserts	8K MWCO	90006	1 plate	Room temperature
		90007	5 plates	
		99006	10 plates	
	12K MWCO	90112	1 plate	
		91012	10 plates	

Required materials not supplied

- Dialysis buffer: for example, phosphate-buffered saline (PBS) containing 100 mM sodium phosphate and 150 mM sodium chloride (Cat. No. 28372)
- Sealing Tape for 96-Well Plates (Cat. No. 15036)

Perform equilibrium dialysis

The Pierce™ RED Device Single-Use Plate is supplied ready to use for dialysis by adding the plasma/compound and buffer to the corresponding chambers. It is not necessary to precondition the membrane inserts. The following is an example protocol, and specific applications and analysis methods can require optimization.

1. For each replicate, prepare samples (50–500 µL) by spiking test compounds with plasma or serum at the appropriate concentrations. To minimize potential errors during sample processing, perform tests in triplicate.
2. Place 50–500 µL of sample into the sample chamber, which is indicated by the colored retainer ring.
3. Add a volume of dialysis buffer to the buffer chamber relative to the sample used as indicated in the following table. Using the appropriate amount of buffer is essential to avoid sample volume changes.

Sample chamber	Buffer chamber
50 µL	300 µL
100 µL	350 µL
200 µL	400 µL
300 µL	550 µL
400 µL	600 µL
500 µL	750 µL

4. Cover the unit with sealing tape and incubate at 37°C on an orbital shaker at approximately 250 rpm or 20 rpm on an up-and-down shaker. Generally, incubating for 4 hours is sufficient to achieve equilibrium; however, actual time required can differ depending on the test compounds and shaker used. For best results, before processing samples perform a test run to empirically determine the time required to reach equilibrium.

   Alternative 100–120 minute procedure: Use an agitation device such as a vortex mixer or shaker that can secure the deep-well plate. Set the mixer at approximately 800 rpm or the shaker at 300 rpm.

   Note: Times greater than 4 hours can be used; however, an excessively long incubation (18 hours) can promote compound instability or result in a sample volume increase from hydrostatic pressure.
5. Remove the sealing tape. Minimal to no volume change should have occurred.
6. Pipette equal volumes from both the buffer and the plasma chambers and place in separate microcentrifuge tubes or into a deep-well plate for analysis. Follow the desired sample preparation procedure for sample analysis.

Analyze samples

Determine the test compound concentrations in the plasma and buffer samples to determine percent bound. Alternatively, compare area ratios against an internal standard between the buffer sample and plasma sample to obtain unbound drug fractions. Some common analysis methods include LC/MS/MS, radioactivity, and UV/visible/fluorescent spectrometry. The following example protocol is for analysis by LC/MS/MS and can be modified if needed.

1. Pipette 25 µL (if the sample used is a 50 µL volume) or 50 µL each of post-dialysis samples from the buffer and the plasma chambers into separate microcentrifuge tubes or plate (Protein Precipitation Plates, Cat. No. 90036 and Cat. No. 90037).
2. Add a corresponding 25 µL or 50 µL of plasma to the buffer sample and an equal volume of buffer to the collected plasma sample.
3. Add 300 µL of Internal Standard containing precipitation buffer (such as cold 90/10 acetonitrile/water with 0.1% formic acid) to precipitate protein and release compound. Vortex and incubate 30 minutes on ice.
4. Centrifuge for 10 minutes at 13,000–15,000 x *g*.
5. Transfer supernatant to a vial or plate for analysis. Alternatively, dry the supernatant and reconstitute before LC/MS/MS.
6. Determine the test compound concentration in the buffer and plasma chambers from peak areas relative to the internal standard. Calculate the percentage of the test compound bound:

   % Free = (Concentration buffer chamber/Concentration plasma chamber) x 100%

   % Bound = 100% – % Free

Additional information

Data comparison

The bound drug (%) in human plasma measured using the Pierce™ RED Device Single-Use Plate on high, medium, and low protein-binding compounds were similar to values reported in the literature using other devices. Compounds were tested at 1 µM.

Table 1 Comparison of results obtained using the RED device with values reported in the literature.

Compound	Human plasma	
	RED plate	Other device[1]
Warfarin	99% bound	99% bound
Taxol	96% bound	95–98% bound
Propranolol	92% bound	80–92% bound
Vinblastine	99% bound	99% bound
Verapamil	90% bound	88–92% bound
Atenolol	4% bound	<5% bound
Antipyrine	0% bound	0% bound

[1] Values reported in the literature (Brouwer et al., 2000; Brunton et al., 2005; Clausen and Bickel, 1993; Sonnichsen and Relling, 1994; Steele et al., 1983).

Related products

Product	Cat. No.	Unit size
Pierce™ Competition RED Device Base Plate	90085	1 base plate body and lid
Pierce™ Competition RED Device Inserts	90087	10 inserts
Pierce™ Protein Precipitation Plates	90036	2 plates
Pierce™ Protein Precipitation Plates	90037	10 plates
Pierce™ RED Device Inserts	89809	50 inserts
Pierce™ RED Device Inserts	89810	250 inserts
Pierce™ RED Device Reusable Base Plate	89811	1 plate
Pierce™ RED Device Single-Use Base Plate	90004	2 plates
Pierce™ RED Device Single-Use Base Plate	90005	10 plates
BupH™ Phosphate Buffered Saline Packs	28372	40 packs
Sealing Tape for 96-Well Plates	15036	100/pkg

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

References

Brouwer EJ et al. (2000) Measurement of fraction unbound paclitaxel in human plasma. *Drug Metab Disposition* 28(10):1141–1145.

Brunton L et al. (2005) *Goodman and Gilman's Pharmacological Basis of Therapeutics* McGraw Hill Publishing: New York.

Clausen J, Bickel M. (1993) Prediction of drug distribution in distribution dialysis and *in vivo* from binding to tissue and blood. *J Pharm Sci* 82:345–349.

Sonnichsen D, Relling M. (1994) Clinical pharmacokinetics of paclitaxel. *Clin Pharmacokinet* 27:256–269.

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* 24:683–687.

Chattoraj DK et al. (1999) Standard free energies of binding of solute to proteins in aqueous medium. Part 2. Analysis of data obtained from equilibrium dialysis and isopiestic experiments. *Biophys Chem* 77:9–25.

Harding SE, Chowdhry B, Eds. (2001) *Protein-Ligand Interactions A Practical Approach Volume 1: Hydrodynamics and Calorimetry* Oxford University Press.

Lin JH, Lu AYH. (1997) Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol Rev* 49:403–449.

Pacifici GM, Viani A. (1992) Methods of determining plasma and tissue binding of drugs. Pharmacokinetic consequences. *Clin Pharmacokinet* 23(6):449–468.

Piafsky KM et al. (1978) Increased plasma protein binding of propranolol and chlorpromazine mediated by disease-induced elevations of plasma alpha1 acid glycoprotein. *N Engl J Med* 299:1435–1439.

Thermo Fisher Scientific | 3747 N. Meridian Road | Rockford, Illinois 61101 USA

For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition. Pierce™ RED Device Single-Use Plate with Inserts are manufactured by Linden Bioscience, Research Triangle Park, NC. US patents issued, 7,604,739 B2 (2009); 8,034,242 B2 (2011).

Revision history: Pub. No. MAN0011619

Revision	Date	Description
C.0	16 June 2023	The format and content were updated.
B.0	26 May 2017	The plate volumes were corrected.
A.0	17 October 2015	New document for the Pierce™ RED Device Single-Use Plate with Inserts.