Pierce™ RED Device Inserts

Catalog Numbers 89809, 89810

Doc. Part No. 2161794 Pub. No. MAN0011571 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 Inserts used along with a required base plate provide an easy-to-use format for equilibrium dialysis experiments. Each insert includes 2 side-by-side chambers separated by an integrated double O-ring-sealed vertical cylinder of dialysis membrane (MWCO approximately 8,000). Two types of the base plate are available: the high-grade Pierce™ RED Device Reusable Base Plate (Cat. No. 89811) is made of durable and chemically inert high-grade PTFE, eliminating nonspecific binding; and the Pierce™ RED Device Single-Use Base Plate (empty) (Cat. No. 90004 and 90005) is disposable and lightweight, allowing routine automation. The Pierce™ RED Device Single-Use Plate with Inserts (Cat. No. 90006 and 90007) is provided with inserts preloaded into the plate.

Equilibrium dialysis is an accurate and reliable method for determining protein binding affinities to chemical or biological substances of low molecular weight. Although the Pierce™ RED Device Inserts are suited for many types of affinity studies, the inserts are specifically designed and extensively validated for plasma serum binding assays and produce results consistent with those reported in the literature (see “Additional information” on page 3). Determining the degree to which a molecule binds to plasma proteins is a critical phase of drug development, as it influences compound dosing, efficacy, clearance rate, and potential for drug interactions.

The design of the Pierce™ RED Device Inserts and base 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. From 1 to 48 Pierce™ RED Device Inserts can be placed into a base plate allowing versatile and cost-effective customization of experiments without unnecessary waste. 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	Cat. No.	Amount	Storage
Pierce™ RED Device Inserts	89809	50 each	Room temperature
	89810	250 each (5 x 50 packs)	

Required materials not supplied

- Pierce™ RED Device Reusable Base Plate (Cat. No. 89811) made of Teflon™ Material or Pierce™ RED Device Single-Use Base Plate (empty) (Cat. No. 90004 or 90005)
- Pierce™ RED Device Insert Removal Tool (Cat. No. 89812) – for easy removal of inserts from the plate
- Dialysis buffer: for example, phosphate-buffered saline (PBS) containing 100 mM sodium phosphate and 150 mM sodium chloride (Cat. No. 28372)
- 20% Ethanol
- Sealing Tape for 96-Well Plates (Cat. No. 15036)

Perform equilibrium dialysis

Prepare the base plate

1. Rinse the base plate wells with 20% ethanol for 10 minutes.
2. Remove ethanol and rinse twice with ultrapure water.
3. Allow the plate to dry. Use the plate immediately, or store the plate covered.

Perform equilibrium dialysis

The Pierce™ RED Device Inserts are supplied ready to use for dialysis with plasma and buffer. Rinsing the insert is unnecessary; however, if rinsing the inserts is desired see "(Optional) Rinse the Pierce™ RED Device Inserts" on page 3. The following example protocol can require optimization for specific applications and analysis methods.

1. For each replicate, prepare the samples by spiking test compounds with plasma or serum at the appropriate concentrations. For best results, test samples in triplicate to minimize potential errors during sample processing.
2. Place inserts open end up into the wells of the base plate. To avoid damage, do not touch the dialysis membrane.

   Note: Place each insert in the same orientation for easy recognition of the sample and buffer chamber.
3. Place 50–500 µL of sample into the sample chamber, which is indicated by the colored retainer ring.
4. 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 maintain the liquid level in both chambers. When using the maximum volumes (i.e., 500 µL (sample chamber) and 750 µL (buffer chamber)), avoid spillover between chambers by using a small pipette tip and handling samples carefully.

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

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

   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: An excessively long incubation (≥18 hours) can promote compound instability or result in a volume increase of the plasma sample from hydrostatic pressure.
6. Remove the sealing tape. Minimal to no volume change should have occurred.
7. 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.
8. Discard the Pierce™ RED Device Inserts and wash/dry the Pierce™ RED Device Reusable Base Plate for future use.

   Note: The inserts are easily removed with forceps or with the Pierce™ RED Device Insert Removal Tool (Cat. No. 89812), which enables fast removal of 8 inserts at one time.

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 the 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 LC/MS/MS analysis and can be modified if needed.

1. Pipette 25 µL (if the sample used is 50 µL in volume) or 50 µL each of post-dialysis samples from the buffer and the plasma chambers into separate microcentrifuge tubes or plates (Protein Precipitation Plates, Cat. No. 90036 and Cat. No. 90037).
2. Add a corresponding 25 µL or 50 µL of plasma to the buffer samples, and an equal volume of buffer to the collected plasma samples.
3. Add 300 µL of 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 the supernatant to a vial or plate for analysis. Add an appropriate internal standard and perform quantitative measurements by LC/MS/MS. Alternatively, dry the supernatant and reconstitute before LC/MS/MS.

   Note: If the final sample is too dilute, dry and reconstitute it before analysis.
6. Determine the concentration of test compound in the buffer and plasma chambers from peak areas relative to the internal standard.
7. 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 percentages of bound drug in human plasma using the Pierce™ RED Device Inserts with the Pierce™ RED Device Reusable Base Plate were similar to values reported in the literature using other devices (Table 1).

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

Compound	RED device	Other device[1]
Ranitidine	17% bound	10–19% bound
Propranolol	84% bound	87–96% bound
Warfarin	99% bound	99% bound
Naproxen	99% bound	99% bound

[1] Values reported in the literature (Jusko and Gretch, 1976; Colangelo et al.,1992; Chan et al., 1994).

(Optional) Rinse the Pierce™ RED Device Inserts

The Pierce™ RED Device Inserts are supplied ready to use for dialysis with plasma and buffer. Rinsing the insert is unnecessary; however, if rinsing the inserts is desired, use the following protocol.

1. Soak the number of required Pierce™ RED Device Inserts in ultrapure water for 10 minutes.
2. Discard water and soak again for 10 minutes. There is no need to remove water from the individual inserts between soaking steps.
3. Store inserts in ultrapure water before use. Do not allow the membranes to dry after rinsing. If required, store inserts in water at 4–8°C for up to 1 week.

Related products

Product	Cat. No.	Unit size
Pierce™ Protein Precipitation Plates	90036	2 plates
Pierce™ Protein Precipitation Plates	90037	10 plates
Pierce™ Competition RED Device Base Plate	90085	1 base plate body and lid
Pierce™ Competition RED Device Inserts	90087	10 inserts
Pierce™ RED Device Reusable Base Plate	89811	1 plate
Pierce™ RED Device Single-Use Base Plate (empty)	90004	2 plates
Pierce™ RED Device Single-Use Base Plate (empty)	90005	10 plates
Pierce™ RED Device Single-Use Plate with Inserts, 8K MWCO	90006	1 plate
Pierce™ RED Device Single-Use Plate with Inserts, 8K MWCO	90007	5 plates
Pierce™ RED Device Single-Use Plate with Inserts, 8K MWCO	99006	10 plates
Pierce™ RED Device Single-Use Plate with Inserts, 12K MWCO	90112	1 plate
Pierce™ RED Device Single-Use Plate with Inserts, 12K MWCO	91012	10 plates
Pierce™ RED Device Insert Removal Tool	89812	1 tool
Acetonitrile	51101	1 L
Trifluoroacetic Acid, Sequanal grade	28904	10 x 1 mL
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

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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™ Rapid Equilibrium Dialysis (RED) Device 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. MAN0011571

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 Inserts.

The information in this guide is subject to change without notice.