

RED Device Inserts

89809 89810

1794.2

Number	Description
89809	RED Device Inserts, 50 each
89810	RED Device Inserts, 250 each (5 × 50 packs)

Storage: Upon receipt store inserts at room temperature.

Introduction

The RED (rapid equilibrium dialysis) Device Inserts used along with the Teflon[®] Base Plate (Product No. 89811) provide an easy-to-use format for equilibrium dialysis experiments. Each disposable insert is comprised of two side-by-side chambers separated by a vertical cylinder of dialysis membrane (MWCO ~8,000). The reusable high-grade Teflon[®] Base is chemically inert eliminating nonspecific binding or risk of contamination.

Equilibrium dialysis is an accurate and reliable method for determining protein binding affinities to chemical or biological substances of low molecular weight (See Pierce website for more detailed information on equilibrium dialysis). Although the 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 Appendix). 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 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 one to 48 RED Device Inserts can be placed into the Teflon[®] 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 × 9 mm well spacing, allowing routine automation. Additionally, the high membrane surface-to-volume ratio allows rapid dialysis, where equilibrium can be reached in 4 hours with high levels of reproducibility and accuracy.

Additional Materials Required

- Teflon[®] Base Plate (Product No. 89811)
- The RED Device Insert Removal Tool (Product 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 (Product No. 28372)
- 20% Ethanol
- Sealing Tape for 96-Well Plates (Product No. 15036)

Procedure for Equilibrium Dialysis

A. Prepare the Teflon Base Plate

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

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B. Equilibrium Dialysis

The 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, refer to the Appendix for the protocol.

The following example protocol may require optimization for specific applications and analysis methods.

1. Prepare 200-500 µl samples by mixing test compounds with plasma or serum at the appropriate concentrations.
2. If the inserts have been pre-rinsed, carefully remove water from each insert by inverting and gently shaking. Place inserts (open end up) into the wells of the Teflon® 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 200-500 µl of sample into the sample chamber, which is indicated by the red ring.
4. Add a volume of dialysis buffer to the buffer chamber relative to sample used as indicated in the table below. Using the appropriate amount of buffer is essential to avoid sample volume changes.

<u>Sample Chamber</u>	<u>Buffer Chamber</u>
200 µl	350 µl
300 µl	500 µl
400 µl	600 µl
500 µl	750 µl

5. Cover the unit with sealing tape and incubate at 37°C on an orbital shaker at approximately 100 rpm or 20 rpm on an up-and-down shaker. Generally, 4 hours of incubation is sufficient to achieve equilibrium; however, actual time required may differ depending on the test compounds. For best results, empirically determine the time required for equilibrium.

Note: An excessively long incubation (≥ 18 hours) may promote compound instability or result in a volume increase in the plasma sample from hydrostatic pressure.

6. Remove seal and confirm volume of the sample chamber. Minimal to no volume change should have occurred.
7. Remove equal volumes from both the buffer and the plasma chambers and place in separate microcentrifuge tubes or into a deep-well plate for analysis.
8. Remove and discard used inserts and wash Teflon Base Plate for reuse.

Note: The inserts can be easily removed with forceps, or the RED Device Insert Removal Tool (Product No. 89812) enables quick removal of eight inserts at once.

Procedure for Sample Analysis

Determine the test compound concentration in the plasma and buffer samples to determine percent bound. Alternatively, compare concentration in the buffer chamber versus a control sample. 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 as needed.

1. Pipette 50 µl each of post-dialysis samples from the buffer and the plasma chambers into separate microcentrifuge tubes or plate.
2. Add 50 µl of plasma to the buffer samples, and an equal volume of PBS to the collected plasma samples.
3. Add 100-200 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 × g.
5. Transfer supernatant to a vial or plate. Add appropriate internal standard and perform quantitative measurements by LC/MS/MS. Alternatively, dry the supernatant and reconstitute before LC/MS/MS.
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 as follows:

$$\% \text{ Free} = (\text{Concentration buffer chamber} / \text{Concentration plasma chamber}) \times 100\%$$

$$\% \text{ Bound} = 100\% - \% \text{ Free}$$

Appendix

A. Data Comparison

The percentages of bound drug in human plasma using the RED Device Inserts with the Teflon[®] Base Plate were similar to values obtained using other devices as reported in the literature (Table 1).

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

<u>Compound</u>	<u>RED Device</u> <u>% bound</u>	<u>Other Device</u> <u>% bound</u>
Ranitidine ¹	17	10-19
Propranolol ²	84	87-96
Warfarin ³	99	99
Naproxen ¹	99	99

B. Rinsing the RED Device Inserts (optional)

The 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 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 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 Pierce Products

51101	Acetonitrile, 1 L
28904	Trifluoroacetic Acid, Sequanal grade, 10 × 1 ml
28372	BupH™ Phosphate Buffered Saline Packs, 40 packs
15036	Sealing Tape for 96-Well Plates, 100/pkg

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2. Colangelo, P.M., *et al.* (1992). Age and propranolol stereoselective disposition in humans. *Clin. Pharmacol. Ther.* **51**:489-94.
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RED Device Products are manufactured by Linden Bioscience, Woburn, MA.

U.S. and international patent pending on RED Device by Linden Bioscience.

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Current versions of product instructions are available at www.piercenet.com. For a faxed copy, call 800-874-3723 or contact your local distributor.

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