INSTRUCTIONS

# RED Device Inserts

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89809 89810

| 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 a required base plate provide an easy-to-use format for equilibrium dialysis experiments. Each insert is comprised of two side-by-side chambers separated by an O-ring-sealed vertical cylinder of dialysis membrane (MWCO ~8000). Two types of the base plate are available: the high-grade Reusable Base Plate (Product No. 89811) is made of durable and chemically inert high-grade PTFE, eliminating nonspecific binding; and the Single-Use RED Base Plate (empty) (Product No. 90004 and 90005) is disposable and light weight, allowing routine automation. Single-Use RED Plates with Inserts (Product No. 90006 and 90007) are provided with inserts pre-loaded 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 (see our 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 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 × 9mm well spacing. 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

- Reusable Base Plate (Product No. 89811) made of Teflon® Material or Single-Use RED Base Plate (empty) (Product No. 90004, 90005)
- 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 100mM sodium phosphate and 150mM sodium chloride (Product No. 28372)
- 20% Ethanol
- Sealing Tape for 96-Well Plates (Product No. 15036)

## Procedure for Equilibrium Dialysis

### A. 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 plate to dry. Use the plate immediately, or store the plate covered.

### B. Equilibrium Dialysis

The RED Device Inserts are supplied ready to use for dialysis with plasma and buffer. Rinsing the insert is unnecessary. The following example protocol may 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 50-500µL 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 table below. Using the appropriate amount of buffer is essential to maintain the liquid level in both chambers. When using the maximum volumes (i.e., 500µL (sample chamber) and 750µL (buffer chamber)), avoid spillover between chambers by using a small pipette tip and handling samples carefully.

| Sample Chamber | Buffer Chamber |
|---|---|
| 50µL | 300µL |
| 100µL | 350µL |
| 200µL | 400µL |
| 300µL | 550µL |
| 400µL | 600µL |
| 500µL | 750µL |

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, ~4 hours of incubation is sufficient to achieve equilibrium; however, actual time required might 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 ~800 rpm or the shaker at 300 rpm.

   **Note:** An excessively long incubation (≥ 18 hours) might promote compound instability or result in a volume increase of the plasma sample from hydrostatic pressure.
6. Remove seal. 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 RED inserts and wash/dry the reusable base plate for future use.

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

## Procedure for Sample Analysis

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 analysis by LC/MS/MS and can be modified as needed.

1. Pipette 25µL (if the sample used is 50µL in volume) or 50µL each of post-dialysis samples from the buffer and the plasma chambers into separate microcentrifuge tubes or plate (Protein Precipitation Plate, Product No. 90036).
2. Add a corresponding 25µL or 50µL of plasma to the buffer samples, and an equal volume of buffer to the collected plasma samples.
3. Add 300µL 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 for analysis. Add 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 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 Reusable 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.**

| Compound | RED Device % bound | Other Device % bound[1-3] |
|---|---|---|
| Ranitidine | 17 | 10-19 |
| Propranolol | 84 | 87-96 |
| Warfarin | 99 | 99 |
| Naproxen | 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 Thermo Scientific Products

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|---|---|
| **89811** | **Reusable Base Plate**, 1 plate |
| **90004** | **Single-Use RED Base Plate (empty),** 2 plates |
| **90005** | **Single-Use RED Base Plate (empty),** 10 plates |
| **90006** | **Single-Use RED Plate with Inserts,** 1 each |
| **90007** | **Single-Use RED Plate with Inserts,** 5 each |
| **89812** | **RED Device Insert Removal Tool** |
| **51101** | **Acetonitrile,** 1L |
| **28904** | **Trifluoroacetic Acid, Sequanal grade,** 10 × 1mL |
| **28372** | **BupH™ Phosphate Buffered Saline Packs,** 40 packs |
| **15036** | **Sealing Tape for 96-Well Plates,** 100/pkg |

### Cited References


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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:**563-9.


### General References


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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.



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




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