INSTRUCTIONS

# Single-Use RED Plate with Inserts

MAN0011619
Rev. B.0
Pub. Part. No. 2162034.7

| Number | Description |
|---|---|
| **90006** | **Single-Use RED Plate with Inserts, 8K MWCO,** 1 each |
| **90007** | **Single-Use RED Plate with Inserts, 8K MWCO,** 5 each |
| **99006** | **Single-Use RED Plate with Inserts, 8K MWCO,** 10 each |
| **90112** | **Single-Use RED Plate with Inserts, 12K MWCO,** 1 each |
| **91012** | **Single-Use RED Plate with Inserts, 12K MWCO,** 10 each |

**Storage:** Upon receipt store inserts at room temperature.

## Introduction

The Thermo Scientific™ Single-Use RED (rapid equilibrium dialysis) Plate is composed of disposable high-density polypropylene and is pre-loaded with 48 equilibrium dialysis membrane inserts. Each insert is comprised of two side-by-side chambers separated by an O-ring-sealed vertical cylinder of dialysis membrane with varying molecular-weight cutoffs (MWCO). This pre-loaded disposable device is automation-friendly, 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 Inserts and Base Plates are also available separately (see Related Thermo Scientific Products Section).

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 detailed information on equilibrium dialysis). The RED Device is specifically designed and extensively validated for plasma serum binding assays and produce results consistent with those reported in the literature (see Appendix). 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 Single-Use RED Plate is validated for minimal nonspecific binding.

The design of the Single-Use RED 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 × 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

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

Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | Thermofisher.com

## Procedure for Equilibrium Dialysis

The Single-Use RED Plate is supplied ready to use for dialysis by simply adding the plasma/compound and buffer to the corresponding chambers. There is no need to pre-condition the membrane inserts. The following is an example protocol, and specific applications and analysis methods might require optimization.

1. For each replicate, prepare samples (50-500µL) 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 50-500µL 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 sample used as indicated in the table below. Using the appropriate amount of buffer is essential to avoid sample volume changes.

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

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 might 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 ~800 rpm or the shaker at 300 rpm.

   **Note:** Times greater than 4 hours can be used; however, an excessively long incubation (≥ 18 hours) could promote compound instability or result in a sample volume increase from hydrostatic pressure.
5. Remove seal. 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.

## 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 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 may be modified as needed.

1. Pipette 25µL (if the sample used is a 50µL volume) or 50µL each of post-dialysis samples from the buffer and the plasma chambers into separate microcentrifuge tubes or plate.
2. Add a corresponding 25µL or 50µL of plasma to the buffer sample and an equal volume of buffer to the collected plasma sample.
3. Add 300µL 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 × *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 as follows:

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

## Appendix

### A. Data Comparison

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

| | Human Plasma (% bound) | |
|---|---|---|
| **Compound** | **RED Plate** | **Other Device*** |
| Warfarin | 99 | 99 |
| Taxol | 96 | 95-98 |
| Propranolol | 92 | 80-92 |
| Vinblastine | 99 | 99 |
| Verapamil | 90 | 88-92 |
| Atenolol | 4 | < 5 |
| Antipyrine | 0 | 0 |

*Values reported in the literature.[1-5]

## Related Products

| | |
|---|---|
| **90085** | **Competition RED Base Plate,** contains base plate body and lid, 1 unit |
| **90087** | **Competition RED Inserts,** contains eight dual- and two single-membrane inserts, 10/pkg |
| **90036** | **Protein Precipitation Plates,** 2 plates |
| **89809** | **RED Device Inserts,** 50 inserts |
| **89810** | **RED Device Inserts,** 250 inserts |
| **89811** | **Base Plate** (made of Teflon™ Material) |
| **90004** | **Single-Use RED Base Plate (empty),** 2 plates |
| **90005** | **Single-Use RED Base Plate (empty),** 10 plates |
| **28372** | **BupH™ Phosphate Buffered Saline Packs,** 40 packs |
| **15036** | **Sealing Tape for 96-Well Plates,** 100/pkg |

### Cited References


1. Brouwer, E.J., *et al.* (2000). Measurement of fraction unbound paclitaxel in human plasma. *Drug Metab Disposition* **28(10):**1141-5.
2. Brunton, L., *et al. Goodman and Gilman's Pharmacological Basis of Therapeutics*. McGraw Hill Publishing: New York, 2005.
3. 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.
4. Sonnichsen, D. and Relling, M. (1994). Clinical pharmacokinetics of paclitaxel. *Clin Pharmacokinet* **27:**256-69.
5. 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.


### General References


Chattoraj, D.K., *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, S.E. and Chowdhry, B., Eds. (2001). Protein-Ligand Interactions A Practical Approach Volume 1: Hydrodynamics and Calorimetry. Oxford University Press.

Lin, J.H. and Lu, A.Y.H. (1997). Role of pharmacokinetics and metabolism in drug discovery and development. *Pharmacol Rev* **49:**403-49.

Pacifici, G.M. and Viani, A. (1992). Methods of determining plasma and tissue binding of drugs. Pharmacokinetic consequences. *Clin Pharmacokinet* **23(6)**:449-68.

Piafsky, K.M., *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**:1435-9.

Products are warranted to operate or perform substantially in conformance with published Product specifications in effect at the time of sale, as set forth in the Product documentation, specifications and/or accompanying package inserts ("Documentation"). No claim of suitability for use in applications regulated by FDA is made. The warranty provided herein is valid only when used by properly trained individuals. Unless otherwise stated in the Documentation, this warranty is limited to one year from date of shipment when the Product is subjected to normal, proper and intended usage. This warranty does not extend to anyone other than Buyer. Any model or sample furnished to Buyer is merely illustrative of the general type and quality of goods and does not represent that any Product will conform to such model or sample.
NO OTHER WARRANTIES, EXPRESS OR IMPLIED, ARE GRANTED, INCLUDING WITHOUT LIMITATION, IMPLIED WARRANTIES OF MERCHANTABILITY, FITNESS FOR ANY PARTICULAR PURPOSE, OR NON INFRINGEMENT. BUYER'S EXCLUSIVE REMEDY FOR NON-CONFORMING PRODUCTS DURING THE WARRANTY PERIOD IS LIMITED TO REPAIR, REPLACEMENT OF OR REFUND FOR THE NON-CONFORMING PRODUCT(S) AT SELLER'S SOLE OPTION. THERE IS NO OBLIGATION TO REPAIR, REPLACE OR REFUND FOR PRODUCTS AS THE RESULT OF (I) ACCIDENT, DISASTER OR EVENT OF FORCE MAJEURE, (II) MISUSE, FAULT OR NEGLIGENCE OF OR BY BUYER, (III) USE OF THE PRODUCTS IN A MANNER FOR WHICH THEY WERE NOT DESIGNED, OR (IV) IMPROPER STORAGE AND HANDLING OF THE PRODUCTS.

Unless otherwise expressly stated on the Product or in the documentation accompanying the Product, the Product is intended for research only and is not to be used for any other purpose, including without limitation, unauthorized commercial uses, in vitro diagnostic uses, ex vivo or in vivo therapeutic uses, or any type of consumption by or application to humans or animals.

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



Pierce Biotechnology 3747 N. Meridian Road | PO Box 117 Rockford, IL 61105 USA | (815) 968-0747 (815) 968-7316 fax | Thermofisher.com