

# Competition RED Device – Inserts and Base Plate

90087 90088 90085

2126.0

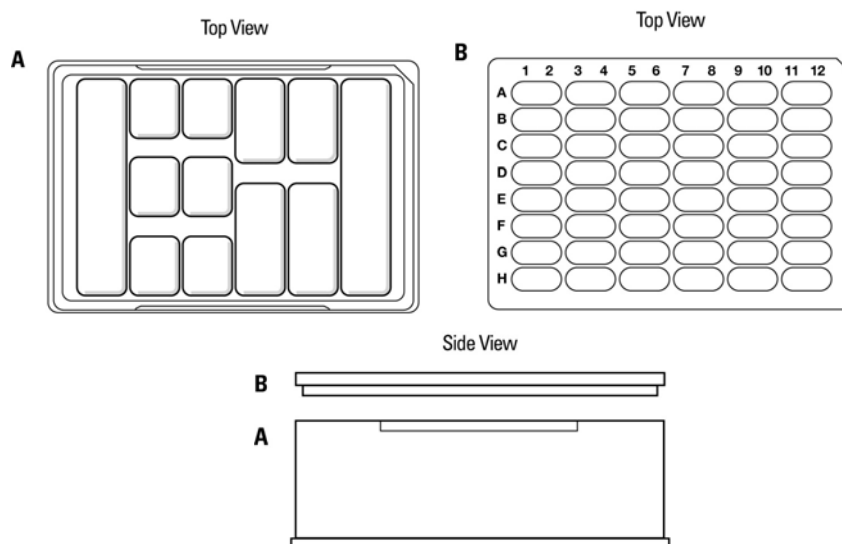
Number	Description
90087	Competition RED Inserts, 10 pack, contains 8 dual-membrane inserts and 2 single-membrane inserts
90088	Competition RED Inserts, 50 pack, contains 40 dual-membrane inserts and 10 single-membrane inserts
90085	Competition RED Base Plate

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

## Introduction

The Thermo Scientific Competition Rapid Equilibrium Dialysis (RED) Device was developed in association with pharmaceutical laboratories to model *in vivo* drug interactions. The inserts used with the Competition RED Base Plate provide an easy-to-use format for equilibrium dialysis experiments involving a small molecule and multiple binding targets. Inserts are comprised of an O-ring-sealed vertical cylinder of dialysis membrane (MWCO ~12,000). The reusable base plate (Product No. 90085), made of high-grade PTFE, is durable and chemically inert, eliminating nonspecific binding.

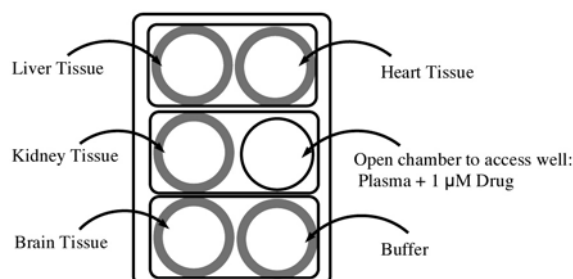
The Competition RED Device requires no extensive assembly or specialized equipment, and each chamber/well is easily accessible from the top of the device. Additionally, the small volume and large surface area allows rapid equilibrium dialysis within 2-4 hours with high levels of reproducibility and accuracy. The base plate enables versatile and cost-effective experiment customization without unnecessary waste. From 4 to 16 dialysis chambers are placed into a common well, and the multi-well format allows several experiments to be conducted simultaneously (Figure 1).



**Figure 1. Format of the Thermo Scientific Competition RED Base Plate.** Panel A: The body of the Base Plate has different well sizes for holding 4, 6, 8 or 16 dialysis chambers (2, 3, 4 or 8 inserts, respectively). The different wells allow a choice of the size best suited for the specific experimental design. Panel B: The lid snaps onto the Base Plate and holds and suspends the dialysis chambers in the wells.

## Important Product Information

- Each 10-pack of Competition RED Inserts contains eight dual-chamber inserts and two single-chamber inserts. The open chamber in the single-chamber inserts enables direct access to the sample in the base plate well without disassembling the device. An example experiment setup for monitoring drug partitioning of buffer, plasma, heart, liver, kidney and brain tissue extracts in a single well is depicted in Figure 2. The buffer chamber is for determining the amount of unbound drug.



**Figure 2. Example experiment setup using a 6-well compartment.**

- The Competition RED Base Plate has several well sizes. Use the smallest well appropriate for the experimental design. The volume of sample required for each well size is listed in Table 1. These volumes assume a well is completely filled with inserts (Figure 2). Add an additional 0.5 ml to a well for each insert slot that is not filled.

**Table 1. Sample volume required for each well size.**

<u>Well</u>	<u>Total Volume*</u>
4-compartment	1.5 ml
6-compartment	2.5 ml
8-compartment	3.0 ml
16-compartment	5.0 ml

\*Add 0.5 ml for each insert slot that is not filled.

- Tissue extract preparation can influence results of equilibrium dialysis experiments. Use the best practices available and use similar procedures when preparing samples for each experiment. General tissue-handling precautions are as follows: do not over-homogenize; dilute extracts 10-fold with an isotonic buffer; and use wide-bore pipette tips.

## Additional Materials Required

- Reusable Competition RED Base Plate (Product No. 90085)
- Isotonic dilution buffer: for example, phosphate-buffered saline (PBS) containing 100 mM sodium phosphate and 150 mM sodium chloride (Product No. 28372)
- Sealing Tape for 96-Well Plates (Product No. 15036)
- 20% ethanol
- Shaker and incubator

## Procedure for Competition Equilibrium Dialysis

### A. Prepare the Base Plate

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

**Note:** The inserts are supplied ready to use for dialysis. Rinsing the insert is unnecessary; however, if you want to rinse the inserts, refer to the Appendix.

## B. Equilibrium Dialysis

The following example protocol is for monitoring the partitioning of a drug between plasma and tissue samples. Optimize or modify this procedure as required for specific applications and analysis methods.

1. Determine the number of binding targets (such as tissue extracts) to be examined in a competition dialysis experiment and the corresponding number of inserts and well size required (see Figure 2 in the Important Product Information section for an example experimental setup).
2. Prepare 10-fold (w/v) tissue homogenates diluted in isotonic dilution buffer. Each dialysis chamber requires 200 µl of extract. Duplicates can be performed in the same well or in a duplicate experiment in another well.

**Note:** If performing precipitation to prepare sample for analysis (see Procedure for Sample Analysis – For Unlabeled Compounds, Step 5) prepare an additional 100 µl of extract.

3. For each test compound, prepare the required volume and concentration by mixing the test compound with plasma diluted 10-fold with isotonic dilution buffer. The volume required is based on the base plate well size (Table 1). For tissue binding studies, a drug concentration of 1 µM is common.
4. Transfer the test compound/plasma mixture prepared in Step 3 to an appropriately sized well. Place lid onto the base plate.
5. Suspend the dialysis chambers (open end up) into the well of the base plate containing the test compound by placing inserts into the appropriate slots of the base plate lid. To avoid damage, do not touch the dialysis membrane.
6. Place 200 µl of tissue extract, buffer or alternative binding target into a dialysis chamber, which is indicated by a red ring. Record the appropriate location of each binding target.
7. Cover the unit with sealing tape and incubate at 37°C on an orbital shaker at 100-500 rpm.

**Note:** Incubating for 4 hours is generally sufficient to achieve equilibrium; however, actual time required may differ depending on the test compounds and shaker used. For best results, perform a pilot experiment to empirically determine the time required to reach equilibrium before processing samples. Time periods greater or less than 4 hours can be used; however, excessively long incubations (i.e., ≥ 6 hours) may promote instability of extracts or test compounds, or result in a volume change from hydrostatic pressure.

8. Remove equal volumes from each of the dialysis chambers and from the bottom well and place them in separate microcentrifuge tubes or into a deep-well plate for analysis. Use an insert with a open side (lacking a dialysis membrane) to directly access the bottom well.
9. Remove and discard used inserts and wash the base plate for reuse.

**Note:** The inserts can be easily removed with forceps or the Thermo Scientific RED Device Insert Removal Tool (Product No. 89812), which enables quick removal of eight inserts at once. Alternatively the base plate lid can be removed and inserts pushed up from the bottom.

## Procedure for Sample Analysis

Determine the test compound concentration in the plasma, buffer and tissue samples to determine the *in vitro* partitioning ratios (K<sub>p</sub>). Some common analysis methods include LC/MS/MS, radioactivity and UV/visible/fluorescent spectrometry. Modify the example protocols below as required.

### • Procedure for radio-labeled compounds

1. Remove 50 µl of each sample and place into a scintillation vial appropriate for available instrumentation. Follow manufacture's recommendations for determining total radioactivity counts per sample.
2. Use the ratio of radio counts to determine the *in vitro* K<sub>p</sub> value between the tissue and the plasma. The free drug fraction can also be obtained from a ratio of the buffer and plasma.

$$K_p = \frac{\text{radio counts of tissue sample}}{\text{radio counts of plasma sample}}$$

$$\text{Free drug} = \frac{\text{radio counts of buffer sample}}{\text{radio counts of plasma sample}} \times 100\%$$

• **Procedure for unlabeled compounds**

**Note:** For unlabeled compounds, place the samples in a common matrix and then treat with precipitation buffer (Step 1) containing an internal standard (IS) before analysis by LC/MS/MS using the protocol below. For fast processing of many samples, use Thermo Scientific Pierce Protein Precipitation Plates (Product No. 90036).

1. Prepare precipitation buffer (e.g., cold 90/10 acetonitrile/water with 0.1% formic acid) containing an internal standard. Each sample requires 600 µl of precipitation buffer.
2. Pipette 50 µl each of the post-dialysis tissue and buffer samples into separate microcentrifuge tubes.
3. Transfer 50 µl each of post-dialysis plasma sample into separate microcentrifuge tubes equal to the number of tissues and buffer samples from Step 2.
4. To each post-dialysis tissue or buffer sample from step 2 add 50 µl of plasma diluted 10-fold with isotonic buffer (i.e., pre-dialysis starting sample minus drug). This creates a consistent matrix between samples to correct for possible variations during precipitation.
5. To the post-dialysis plasma samples from Step 3, add 50 µl of a corresponding tissue extract (i.e., pre-dialysis starting sample) to create a consistent matrix between samples to correct for possible variations during precipitation.

**Note:** Refer to Table 2 for an example of steps 3-5 for the experiment represented in Figure 2.

**Table 2. Example precipitation setup.**

<u>Tube #</u>	<u>Sample (50 µl)</u>	<u>Matrix (50 µl)</u>
1	Post-dialysis liver extract	10X diluted plasma
2	Post-dialysis heart extract	10X diluted plasma
3	Post-dialysis kidney extract	10X diluted plasma
4	Post-dialysis brain extract	10X diluted plasma
5	Post-dialysis buffer	10X diluted plasma
6	Post-dialysis plasma	liver extract
7	Post-dialysis plasma	heart extract
8	Post-dialysis plasma	kidney extract
9	Post-dialysis plasma	brain extract
10	Post-dialysis plasma	isotonic buffer

6. Add 300 µl of precipitation buffer to each tube. Vortex and incubate for 30 minutes on ice. Precipitation of the proteins in the sample releases free and bound drug for analysis.
7. Centrifuge for 10 minutes at 13,000-15,000 × g.
8. Transfer supernatant to a vial or plate for quantitative measurements by LC/MS/MS.

**Note:** If final sample is too dilute, dry and reconstitute it in a smaller volume before analysis.

9. Determine the concentration of test compound in the buffer and plasma chambers from peak areas relative to the internal standard.
10. After LC/MS/MS analysis, calculate the *in vitro* K<sub>p</sub> value as follows:

$$K_p = \frac{\text{sample count ratio against IS}}{\text{plasma count ratio against IS}}$$

$$\text{Free drug} = \frac{\text{buffer count ratio against IS}}{\text{plasma count ratio against IS}} \times 100\%$$

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## Appendix

### A. 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 you want to rinse the inserts, use the following protocol.

1. Soak the number of required Competition RED Device Inserts in ultrapure water for 10 minutes.
2. Discard water and soak 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.
4. Before use remove water by inverting and shaking gently.

### Related Thermo Scientific Products

15036	Sealing Tape for 96-Well Plates, 100/pkg
28372	BupH™ Phosphate Buffered Saline Packs, 40 packs
51101	Acetonitrile, 1 L
28904	Trifluoroacetic Acid, Sequanal grade, 10 × 1 ml
90036	Pierce Protein Precipitation Plates
89809	RED Device Inserts, 50/pk
90004	Single-Use RED Base Plate (empty), 2 plates

RED Device Products are manufactured exclusively for Thermo Fisher Scientific by Linden Bioscience.  
U.S. and international patent pending on RED Device by Linden Bioscience.

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