

# Automated Rapid Equilibrium Dialysis using Thermo Scientific Matrix PlateMate Automation

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## Abstract

The Thermo Scientific Pierce Rapid Equilibrium Dialysis (RED) is a rapid and efficient device for conducting dialysis. Configured in a 96-well format and compatible with automation; it helps reduce sample wastage and user involvement. In this study, we demonstrate RED compatibility with the Thermo Scientific Matrix PlateMate 2x3 automated liquid handling workstation. We present a protocol for using this instrument to deliver dispenses into 96-well plates which serve to model the RED apparatus. Our findings indicate that the PlateMate 2x3 enables reliable and efficient tip access and liquid dispenses into the RED apparatus.

## Introduction

Throughout the duration of drug development, new drugs are thoroughly screened in an extensive series of regulated tests which permit researchers to determine the effects, clearance rate, and interactions of a drug. Equilibrium dialysis is a common screening method for separating smaller molecules (i.e. drug) from larger molecules (i.e. protein) by selective diffusion through a semipermeable membrane. It is often used to approximate the nonbound drug fraction in plasma. As a result of this screening, drug binding affinity, multiple-drug interactions, and effective drug dosage can be predicted.

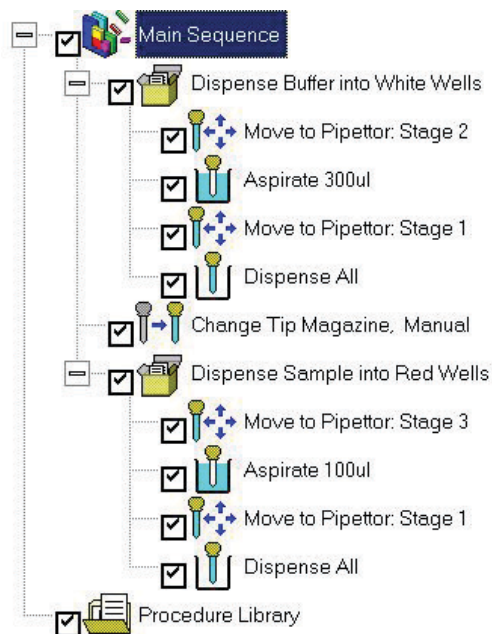
Traditional dialysis is a time-consuming and laborious process that necessitates multiple buffer changes and a large initial sample volume. Most often, the sample is diminished as a result of operational loss and sample attrition. The RED apparatus was created to reverse the setbacks of this process. It can hold as little as 100  $\mu\text{l}$ /well of sample and requires no buffer changes, user-intervention or supervision. Moreover, it can accomplish rapid dialysis in a minimal 4-hour time frame. Here, we describe a protocol for using the Thermo Scientific Matrix PlateMate 2x3 to deliver accurate dispenses into 96-well plates. We will substitute the RED device with 96-well plates to allow spectrophotometric measurement and analysis of program efficiency.

## Automation

The Thermo Scientific Matrix PlateMate 2x3 workstation is a versatile liquid handling platform configurable with eight disposable or fixed tip pipetting heads with a 0.1-300  $\mu\text{l}$  combined volume range. These



**Figure 1: The Rapid Equilibrium Dialysis (RED) Apparatus. RED is composed of alternating rows of red cylindrical and white conical wells that are mutually linked to allow buffer entry into dialysis chamber.**



**Figure 2: This ControlMate program sequence is used to deliver dispenses into the RED device. The program was validated with reagent solution dispenses into 96-well plates and with a dry run using the RED Device.**

are ideal for applications requiring disposable tips, serial dilutions and procedures requiring high aspiration volumes. The PlateMate 2x3 has high resolution X-Y plate positioning (0.06mm) to ensure reliable tip access into

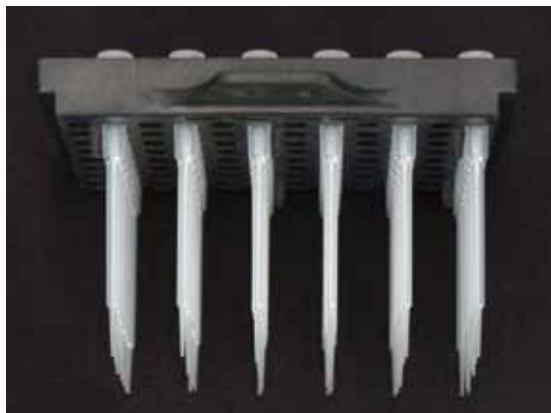
96-, 384-, and 1536-well plates as well as into a multitude of other accessories. The PlateMate 2x3 functions are controlled by Thermo Scientific ControlMate software which facilitates the simple creation of intricate applications using drag-and-drop icons.

### Rapid Equilibrium Device

The RED Device System uses tube inserts made of two side-by-side chambers separated by a vertical cylinder of dialysis membrane. The inserts form alternating columns of red cylindrical and white conical wells (Figure 1). Red wells serve as sample chambers while white wells hold buffer solution. The chambers are mutually linked to permit buffer entry into sample chamber. The membrane has an 8000 Dalton cut-off mass enabling the successful separation of proteins from smaller molecules. Our experiment requires the use of an extended length tip magazine to prevent tip contact with wells because the well orifice of the RED apparatus is smaller than the standard well diameter of a 96-well plate.

### Materials

1. Thermo Scientific Matrix PlateMate 2x3 (Item No. 801-10005)
2. Thermo Scientific Matrix D.A.R.Ts Tips, 96-format, 300  $\mu$ l, Extended Length (Item No. 5536)
3. Thermo Scientific Pierce Rapid Equilibrium Dialysis (RED)
4. Thermo Scientific Matrix Air Displacement Pipetting Head, 96 Channel, 5.0-300  $\mu$ l (Item No. 501-20001)
5. Albumin Fraction V source: Bovine Blood
6. Tartrazine Dye
7. Glycerol
8. Thermo Scientific Pierce BupH Phosphate buffered saline pack diluted in 500 mL distilled water pH 7.4
9. Centrifuge
10. Plate Reader



**Figure 3:** An example of the tip magazine arrangement. Tips are arranged in an alternating column order to dispense only into red or white wells.

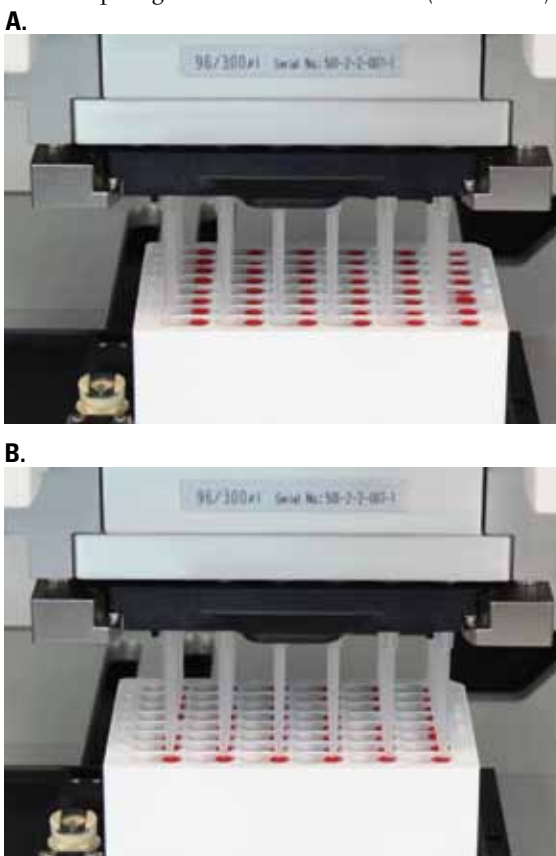
### Method

#### RED Device Setup:

In order to confirm program effectiveness, a dry run, a run without using liquids was executed using the RED apparatus with two dye solution reservoirs placed onto stage positions 3 and 4 (Figure 4). Tips were arranged in an alternating column order to dispense into either red columns or white columns (Figure 3). Stage set-up and labware positions are indicated in figure 4.

Tip heights were adjusted to prevent air-bubble formation in the bottom of the wells and to decrease the probability of tip contact with the membrane. Contact could result in a membrane puncture which could produce sample cross-talk and fractional dialysis.

- A. Tip height for red wells: 8550 (1/100 mm)
- B. Tip height for white wells: 8500 (1/100 mm)



**Figure 4:** The following images present the Thermo Scientific PlateMate platform delivering dispenses into the white columns image A, and into the red columns image B, of the RED device. After dispensing into the white wells, the tip magazine is changed and tips are rearranged in an opposite alternating column order to enable a direct dispense cycle into the red wells.

#### Dispensing into 96-Well Plates:

1. Reagent Solution was made using 50 mg/mL Bovine Albumin in 0.05% tartrazine dye and PBS solution. (Note: Albumin concentration in human plasma is 30-50 mg/mL)
2. An empty 96-well plate was placed onto position 1 and two reservoirs with Reagent Solution were placed in positions 3 and 4.

- Using the program sequence in Figure 2 “Dispense Buffer into White Wells” 300 µl/well of Reagent Solution was dispensed into a 96-well plate filling all the wells.
- The plate was removed and replaced with a fresh 96-well plate.
- Subsequently, the following sequence “Dispense Sample into RED Wells” was run to dispense 100 µl/well of Reagent Solution into the new plate.
- Both plates were spun at 1000 rpm and read at 412 nm absorbance.

## Results

In this study, the Thermo Scientific Matrix PlateMate automated liquid handler was combined with a 0.5-300 µl air-displacement head and an extended length 5.0- 300 µl D.A.R.T.s tip to execute a program for dispensing solution into the wells of the Rapid Equilibrium Dialysis Device (RED). The PlateMate 2x3 was tested to deliver precise dispenses into two 96-well plates, filling each plate entirely. Plate readings yielded a coefficient of variance (% CV) of 1% for the 300 µl dispense, and 2% for the 100 µl dispense. Furthermore, the program was executed using the RED apparatus, and was efficiently completed with no tip-to-well contact. Hence, PlateMate automation can be used with the RED device to perform reliable device set-up for rapid equilibrium dialysis.

**Table 1: Program Optimizations and Results**

Sample Buffer	Tip Height (1/100 mm)	Dispense Volume	Average	Standard Deviation	% Coefficient of Variance
0.05% Dye, Albumin, PBS	8500	300 µl	0.9766	0.008821	1%
0.05% Dye, Albumin, PBS	8550	100 µl	0.3025	0.005774	2%

## Conclusion

Results indicate that Thermo Scientific Matrix PlateMate 2x3 is an ideal platform on which to perform RED device setup and is recommended for high throughput facilities that require a simple and time-restrained procedure for sample dialysis.

## References

Rapid Equilibrium Dialysis (RED) Device. A Transforming Technology for Plasma Protein Binding Assays. Thermo Scientific Pierce Research Products. Thermo Scientific. May 1, 2009. [www.piercenet.com](http://www.piercenet.com)

Susan Chen, Ji Zhang, Tai-Nang Huang<sup>1</sup>, Jing-Tao Wu, Frank W. Lee, and Mark G. Qian.” A Practical Method for Measuring Free Drug Concentration in Whole Blood Using an Equilibrium Dialysis Method.” Thermo Scientific Pierce Research Productions. October 12, 2007. May 1, 2009. [www.piercenet.com](http://www.piercenet.com)

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