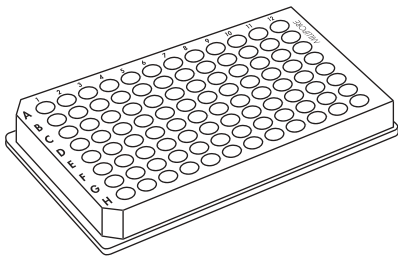


# **MultiScreen® Filter Plates with Ultracel™-PPB Membrane**

**For use in the separation of serum  
protein-bound drug from free drug in  
plasma protein binding assays**

**For research use only.  
Single use only.**



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## Introduction

The MultiScreen filter plate with Ultracel-PPB membrane is a single-use, 96-well assembly that includes a filter plate, filtrate receiver plate, and an extended centrifugal cover. The device is intended for the processing of serum or plasma samples in the 0.1 to 0.5 mL volume range. The device is for use in a centrifugal pressure mode only, and is compatible with standard centrifuge microtiter plate swinging bucket rotors. It is designed to fit with a standard 96-well microtiter receiver plate for use in ultrafiltrate collection.

The MultiScreen filter plate with Ultracel-PPB membrane has been developed and is QC-released for use in plasma or serum protein binding assays for purposes of determining the extent of protein bound or free compound or drug. The low binding ultrafiltration membrane used in this product has a 10,000 Dalton nominal molecular weight limit (NMWL) as determined with the protein solute marker cytochrome C (12,500 Daltons). Serum proteins such as albumin will be 99.5% retained.

The MultiScreen filter plate with Ultracel-PPB membrane is packaged fully assembled with the 96-well filtrate collection plate and an extended centrifugal cover which eliminates risk of sample evaporation during centrifugation.

## Materials Required

### *Included*

- MultiScreen filter plate with Ultracel-PPB membrane assembly (see Figure 1). Microtiter plate storage lids are also included.

### *User Supplied*

- Centrifuge capable of a minimum of 2000 x g-force with a swinging bucket rotor and 96-well plate carrier. **Do not use this plate with vacuum filtration.**
- Pipettors or robotic liquid handlers for transferring 300  $\mu\text{L}$  and 25  $\mu\text{L}$  and 10  $\mu\text{L}$  volumes.
- Analytical instrumentation for determining drug concentration (e.g., HPLC, LC/MS/MS)

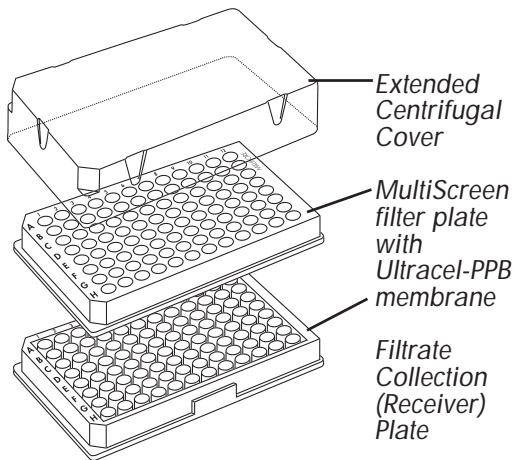


Figure 1. MultiScreen filter plate with Ultracel-PPB membrane assembly.

## Specifications, Limitations and Precautions

### *Specifications*

Filter plate well capacity	500 $\mu$ L
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Receiver microtiter plate well capacity	300 $\mu$ L
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Working sample volume capacity

with included receiver plate	300 $\mu$ L
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with a deep well plate (not included)	500 $\mu$ L
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*Note: Serum samples do not spin to dryness, and up to 500  $\mu$ L can be spun in the MultiScreen Ultracel-PPB assembly.*

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Centrifugal Speed

Maximum	3000 x g
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Recommended	2000 x g
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Dimensions, assembled plate

Length	128.6 mm
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Width	86 mm
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Depth	29.4 mm
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Materials of Construction

Filter plate	Polyolefin
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Membrane	10,000 NMWL
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	Regenerated Cellulose
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### *Limitations and Precautions*

- Centrifuge at a temperature range of 25 to 37 °C. Plasma protein drug or compound binding is normally determined at 37 °C.
- Complex samples such as serum or plasma can be difficult to filter (relative to other biological samples). The recommended g-force and spin time conditions have been established with a range of serum and plasma types. The ultrafiltrate volumes reported in this guide are based on the recommended operating conditions and represent the minimum volume generated in any one well. The composition of serum or plasma depends on type, source, and whether it is fresh or previously frozen. Filtration of fresh plasma can be unpredictable and the operating conditions described here may result in lower minimum ultrafiltrate volumes.
- The MultiScreen filter plate with Ultracel-PPB membrane may exhibit a variance in flow properties with viscous, particle-laden samples such as serum and plasma. Depending on the centrifuge, wells on the outside edges (i.e., rows A, B, G, and H) of the filter plate will tend to filter slightly faster than those on the inside of the plate. This will NOT

*Limitations and Precautions*, continued

interfere with data analysis or assay results (see table 1 and figure 2).

- This product is not recommended for use with dilute biological samples in starting volumes of greater than 300  $\mu\text{L}$ , because the sample may be filtered entirely and the receiver plate will not be able to contain the entire ultrafiltrate volume. For samples exceeding 300  $\mu\text{L}$ , use of a deep well plate will ensure that the receiver plate will contain all of the ultrafiltrate.
- The serum or plasma ultrafiltrate volume generated can be increased by using a larger starting volume of sample. Use of a 500  $\mu\text{L}$  sample of serum or plasma (versus recommended 300  $\mu\text{L}$ ) will generate a larger ultrafiltrate volume (See Figure 3).
- MultiScreen Ultracel-PPB plates are designed for centrifugal pressure mode only. **Do not use this plate with vacuum filtration.**

*Limitations and Precautions*, continued

- Centrifuge microtiter plate carriers (plate bucket) must be flat to provide uniform support during centrifugation. If the carrier base is not flat, performance may be compromised.
- To minimize evaporation of sample prior to analysis and during centrifugation, the top of the MultiScreen filter plate with Ultracel-PPB membrane should be covered with the extended centrifugal cover.

Row	Column											
	1	2	3	4	5	6	7	8	9	10	11	12
<b>A</b>	74	73	72	69	61	61	69	71	73	63	71	66
<b>B</b>	64	62	56	68	60	69	55	54	56	58	59	61
<b>C</b>	55	51	51	54	54	51	55	49	49	48	49	53
<b>D</b>	53	47	52	59	50	48	45	45	41	40	42	43
<b>E</b>	55	52	49	55	55	49	46	43	50	46	48	44
<b>F</b>	65	60	77	75	55	57	55	50	60	55	61	54
<b>G</b>	68	62	65	69	64	72	65	62	72	66	70	66
<b>H</b>	81	65	70	75	70	70	70	79	74	86	75	73

*Table 1. Typical filtrate volume ( $\mu\text{m}$ ) results for Multiscreen plate with Ultracel PPB membrane at 2000 x g, 37 °C, 45 minute spin, with once frozen human plasma.*

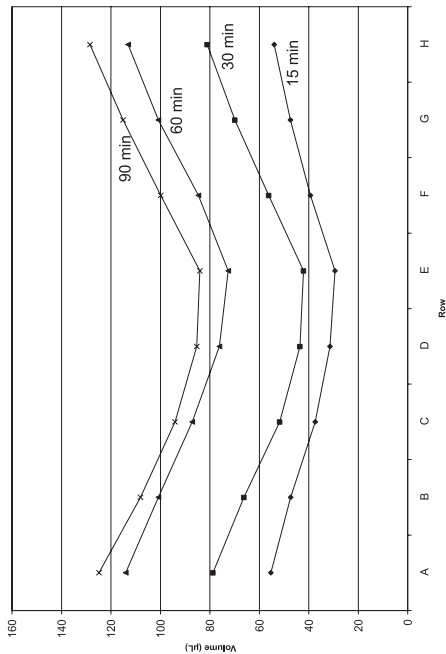


Figure 2. Serum ultrafiltration volume versus well position for a starting volume of 300 µL of human serum centrifuged at 2000 x g and 37 °C for various centrifuge spin times.

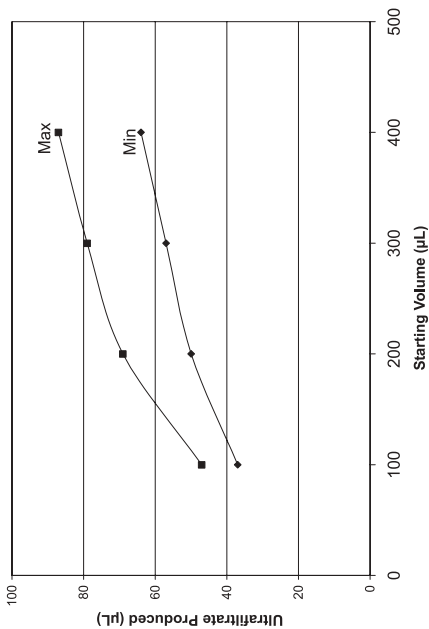


Figure 3. Starting volume versus ultrafiltrate produced for human serum with a 60 minute centrifuge spin time at 2000 x g and 37 °C.

## **Operation and Performance Guidelines**

The MultiScreen filter plate with Ultracel-PPB membrane is operated in a centrifugal pressure mode at a recommended temperature of 37 °C and g-force of 2000 x g. When mounted on top of a microtiter receiver plate the MultiScreen filter plate with Ultracel-PPB membrane will fit into all standard microtiter plate swinging bucket rotors.

Drug concentration in the ultrafiltrate can be determined by a variety of methods with the most common being **Liquid Chromatography/Mass Spectrometry (LC/MS)** or a radiometric method with **Liquid Scintillation Counting (LSC)**.

## *General Operating Procedure*

1. Add drug from a concentrated solution to plasma or serum in an appropriate container or well of a multiwell device to achieve a desired final concentration of drug. **Do not use the MultiScreen filter plate with Ultracel-PPB membrane as a mixing vessel.**
2. Cover the container and incubate for 1 hour at 37 °C.
3. Load samples into the MultiScreen filter plate with Ultracel-PPB membrane using a standard multi-channel pipetter or liquid handling instrument.
4. Assemble the MultiScreen filter plate with Ultracel-PPB membrane, filtration collection plate and the extended centrifugal cover.
5. Load the assembly into the centrifugal microtiter plate carrier and centrifuge at 2000 x g for 45 minutes. Refer to Table 2 and Figure 4 for additional information regarding centrifugation guidelines for different spin times and sample types. Starting with more volume will increase the volume of ultrafiltrate generated. The wells of the MultiScreen filter plate with Ultracel-PPB membrane can handle up to 500 µL of serum.

Spin Time (min)	300 $\mu$ L Initial Volume		500 $\mu$ L Initial Volume	
	Final Filtrate Volume ( $\mu$ L)		Final Filtrate Volume ( $\mu$ L)	
	Min	Max	Min	Max
15	30	55	35	60
30	45	80	55	95
60	75	115	75	120
90	85	130	85	140

*Table 2. Final filtrate volumes for 300  $\mu$ L and 500  $\mu$ L starting volumes of human serum at 2000 x g, 37 °C, 45 minutes.*

6. To recover the sample ultrafiltrate, remove the Ultracel-PPB filter plate from the microtiter receiver plate and pipette the desired volume from each well of the filtrate receiver plate for analysis.
7. Analyze the drug concentration in the ultrafiltrate to determine plasma protein binding. Refer to the Data Analysis section for the percent plasma protein binding (PPB) and mass balance (MB) calculations.

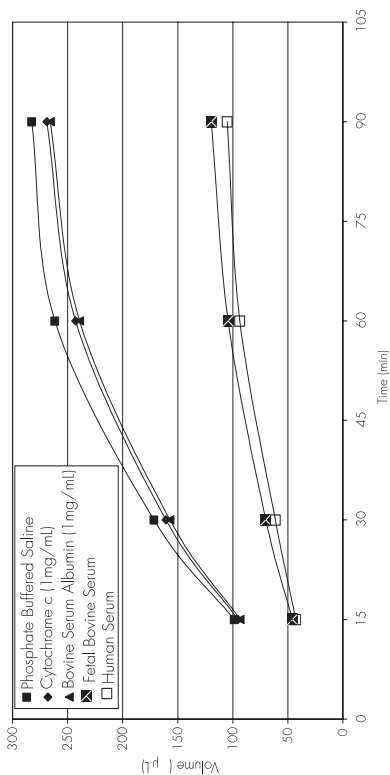


Figure 4. Typical filtrate volume versus spin time. This graph shows the effect of 2000 x g centrifuge time on ultrafiltrate volume for different media with an initial sample volume of 300 µL. Ultrafiltrate volumes are lower for samples with higher protein concentrations such as serums.

## Mass Balance for Method Validation

Since loss of free drug may occur due to variations in sample preparation methods, metabolism or non-specific binding (NSB), PPB studies may include the determination of mass balance (MB) to establish good overall drug recovery. MB is the ratio of mass of the drug in the retentate plus the filtrate divided by the total mass of drug added in the starting volume. The drug mass balance is calculated by determining volumes of and drug concentrations in both the retentate and the ultrafiltrate. When removing the retentate aliquot ensure that the retentate is well mixed as a concentrated protein layer may form on the membrane during filtration. Analyze the remaining ultrafiltrate by methods that include gravimetric, dilution factor of a chromophore or near infrared. Please contact Technical Services for additional information.

### *Data Collection*

Drug concentrations are determined for the ultrafiltrate and for the retentate if mass balance will be done. For good quantitative analysis, standard curves should be made. Dilutions of each drug solution are prepared using three sequential 10X dilutions in PBS (e.g., 5000, 500, 50, 5 nM). Care should be taken to account for difference in media on the analysis. Mass balance

analysis requires the determination of filtrate volumes. Contact Technical Services for additional information.

### *Data Analysis by Percent Drug Plasma Protein Binding Determination*

After the concentration of free drug and total drug has been determined by any number of validated methods including LCS and LC/MS, percent plasma protein drug binding can be calculated as follows:

$$\% \text{ Protein Binding} = \left( 1 - \frac{[\text{drug}_{\text{ultrafiltrate}}]}{[\text{drug}_{\text{total}}]} \right) \times 100\%$$

### Mass Balance Determination

After filtrate volume, concentration of free drug, drug in the retentate and total drug have been determined by any number of validated methods including LSC and LC/MS, mass balance can be determined as follows:

$$\text{Mass Balance} = \left( \frac{\text{Mass}_{\text{ultrafiltrate}} + \text{Mass}_{\text{retentate}}}{\text{Mass}_{\text{total, initial}}} \right) \times 100\%$$

$$\text{Mass Balance} = \left( \frac{D_{\text{ultrafiltrate}} \times V_{\text{ultrafiltrate}} + D_{\text{retentate}} \times V_{\text{retentate}}}{D_{\text{initial}} \times V_{\text{initial}}} \right) \times 100\%$$

Where: MB = Mass Balance  
 D = Drug Concentration  
 V = Volume  
 $V_{\text{retentate}} = V_{\text{initial}} - V_{\text{ultrafiltrate}}$

## *Results*

Standard curves were fit with a quadratic equation and then this equation was used to calculate drug concentration from the observed counts for each analysis. PPB and MB values (Table 3) were determined based on these concentrations and the volumes determined by the Dilution Factor Method. Please contact Technical Services for additional information.

The percent plasma protein binding values for all of the drugs determined with the MultiScreen filter plate with Ultracel-PPB membrane over several days with different plates and solutions are all in agreement with reported values. Additionally, the coefficient of variance for these determinations ranged from approximately 5% (for methotrexate) to 1% (for propranolol) to <1% for warfarin.

Chemical Name	Plasma Protein Binding			Mass Balance		Number of Analysis
	Published Range <sup>1</sup>	ave.	s.d.	ave.	s.d.	
caffeine	29-43	37%	4%	106%	7%	81
methotrexate	35-57	39%	5%	93%	7%	82
propranolol	81-93	84%	1%	102%	5%	40
testosterone	85-95	95%	1%	103%	14%	42
warfarin	98-100	99%	0.4%	96%	18%	83

*Table 3. Percent Plasma Protein Binding for Methotrexate, Propranolol, and Warfarin determined using the MultiScreen filter plate with Ultracel-PPB membrane. 300  $\mu$ L of 5  $\mu$ M drug in human plasma incubated at 37 °C for one hour.*

## Quality Assurance/Product Release Criteria

- **Filter Plate Dimensions/Tolerances**  
All components meet SBS dimensional guidelines.
- **Protein Retention**  
The membrane retention (10,000 NMWL) is greater than 99.5% for serum albumin in 50% (40 mg/mL concentration) adult bovine serum. Protein retention at this level will yield excellent plasma protein binding results for low, medium and high binding drugs (See Results Section).
- **Minimum Filtrate Volume**  
The minimum filtrate volume in each well is greater than 45  $\mu$ L following centrifugation of 200  $\mu$ L of 50% adult bovine serum at 3000 x g for 30 minutes at 37 °C. Some variability in the filtrate volume across the plate may be observed. Correlation between filtrate volume and PPB is negligible. Please see Results Section for more details.
- **Drug Recovery**  
The materials of the plate have been qualified for high drug recoveries or low non-specific binding (NSB) and all new lots are tested with a panel of 10

different drugs to confirm this and ensure high drug recovery. Please contact Technical Services for additional information.

## **References**

<sup>1</sup> Goodman, L.S., et al., “Goodman & Gilman’s The Pharmacological Basis of Therapeutics”. 10th edition. 2001, New York: McGraw-Hill Medical Pub. Division. xxvii, 2148.

## **Technical Service**

For more information, contact the Millipore office nearest you. In the U.S., call **1-800-MILLIPORE** (1-800-645-5476). Outside the United States, see your Millipore laboratory catalogue for the phone number of the office nearest you. You can also look us up on the Internet at [www.millipore.com](http://www.millipore.com).

When making inquiries regarding product performance or applications, please provide the product lot number and catalogue number located on the package bar code label.

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