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# *Technical Note*

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## A Publication of Technical Services

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**Title:**           **Drug Solubility Assay using the 96-well MultiScreen<sup>®</sup>  
Solubility filter plate on the Beckman Biomek<sup>®</sup> FX  
Workstation**

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### **Introduction**

Determining compound solubility has become an essential tool in the early stages of the drug discovery process. Low solubility can lead to unreliable results during in-vitro testing. Also, insoluble precipitates have been shown to cause false positives in bioassays, wasting valuable time and resources. Such issues will typically add significant cost to drug research projects. In addition to these factors, the standard shake-flask method used to evaluate drug solubility is inherently low throughput and labor intensive.

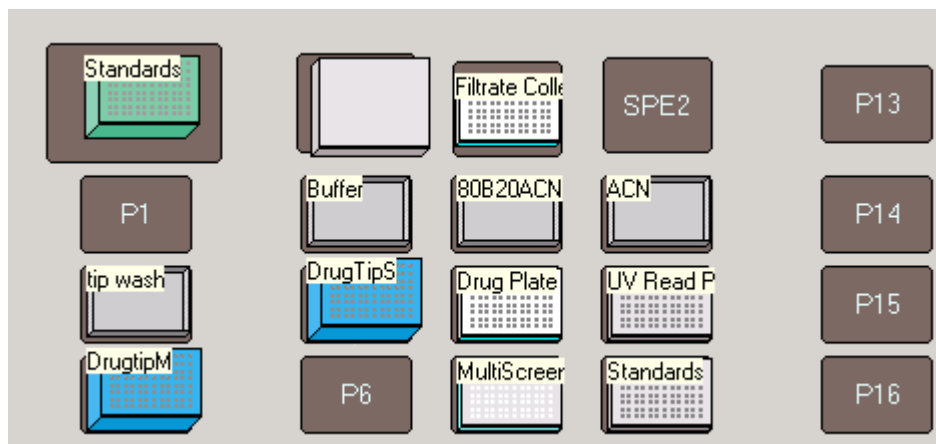
Here we describe an automated method for determining drug solubility using Millipore's MultiScreen Solubility plate on the Biomek FX workstation. The procedure requires minimal manual intervention by utilizing the gripper for all plate movements on the deck, but shaking occurred offline. It takes about 1 hour and 45 minutes to process on plate of 96 samples (this includes 90 minute shaking off-line). Eight compounds were tested in nine replicates and the results were compared to those achieved through the manual method to demonstrate the viability of the automated protocol. For quantification of aqueous solubility, it is recommended that a standard calibration curve be completed and analyzed for each compound prior to determining aqueous solubility (refer to protocol note PC2445EN00).

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## Configuration of the Biomek FX Deck Setup



### Prior to starting program, make sure the deck configuration is as follows:

- TL1: P200 tips (replace with fresh box after shaking offline).
- P1: Empty (at the beginning)
- P2: Wash station (upside down tip box) for tip washing.
- P3: P20 tips for transferring drug from drug plate to MultiScreen plate.
- Holder1: Millipore manifold collar.
- P4: Universal buffer (pH 7.4) reservoir.
- P5: P20 tips for transferring drug from drug plate to standards plate.
- P6: Empty.
- SPE1: Millipore manifold with v-bottom plate for collecting filtrate.
- P7: 80% Universal buffer/20% Acetonitrile reservoir.
- P8: V-bottom plate with drug compounds (10 mM in DMSO).
- P9: MultiScreen Solubility Plate.
- P10: 100% Acetonitrile reservoir.
- P11: UV plate for sample analysis.
- P12: UV plate for standards.

\*Refer to application notes AN1730EN00 or AN1731EN00 for description of solution preparation.

**Procedure:**

1. Distribute 190  $\mu$ L aliquots of the Universal buffer (P4) to the MultiScreen Solubility plate (P9).
2. Wash tips in DIW water, 10 times (P2) and unload tips.
3. Add 10  $\mu$ L of drug compound from the v-bottom plate (P8) to the MultiScreen Solubility plate (P9) using P20 tips (P3).
4. Distribute 192  $\mu$ L of the 80% Universal buffer/20% Acetonitrile (P7) to the standards UV plate (P12) using P200 tips.
5. Add 8  $\mu$ L of drug compound from the v-bottom plate (P8) to the standards UV plate (P12) using P20 tips (P5).
6. Mix the standards plate (P12) 10 times using the P200 tips.
7. Manually remove standards UV plate (P12) and the MultiScreen Solubility plate (P9).
8. Cover the MultiScreen Solubility plate and shake at 300 rpm for 90 minutes (offline if a Biomek shaker is not available).
9. Return the MultiScreen Solubility plate (uncovered) to position P9. Place a fresh box of P200 tips at TL1 and click okay.
10. Gripper places the Millipore manifold collar on-top of the filtrate collection plate at SPE1.
11. Gripper places the MultiScreen Solubility plate (P9) on top of the manifold.
12. Filter at 9" Hg for 1 minute.
13. Move MultiScreen Solubility plate to P9. Disassemble SPE manifold and move the filtrate collection plate to P12.
14. Transfer 160  $\mu$ L of the filtrate from the collection plate (P12) to the UV analysis plate (P11) using P200 tips (TL1).
15. Distribute 40  $\mu$ L of Acetonitrile (P10) to all wells of the UV analysis plate (P11) using P200 tips.
16. Manually remove the UV analysis plate and cover. Shake at 300 rpm for 5 minutes.

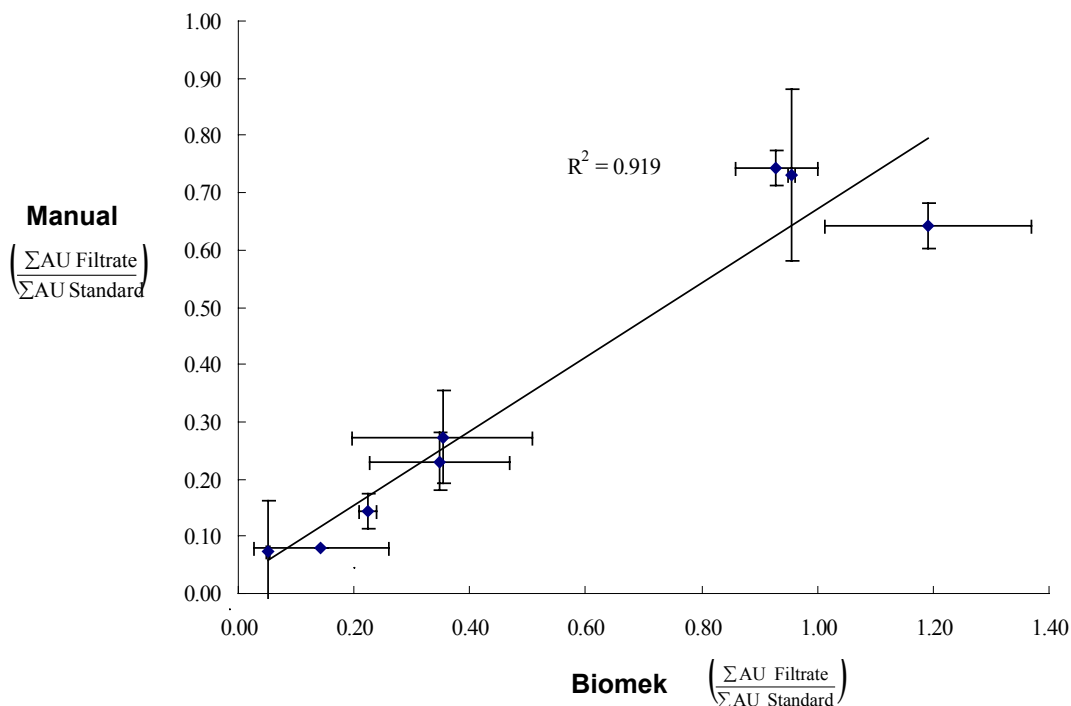
**Table 1. Drug solubility**

Drug	Biomek Automation Method		Manual Method	
	$\left( \frac{\sum \text{AU Filtrate}}{\sum \text{AU Standard}} \right)$		$\left( \frac{\sum \text{AU Filtrate}}{\sum \text{AU Standard}} \right)$	
Sample	mean	Standard deviation	mean	Standard deviation
4,5 DPI	0.22	0.02	0.14	0.03
benzanthrone	0.05	0.00	0.07	0.09
$\beta$ -estradiol	0.14	0.12	0.08	0
diethylstilbestrol	0.35	0.16	0.27	0.08
griseofulvin	0.93	0.07	0.74	0.03
ketoconazole	0.35	0.12	0.23	0.05
phenazopyridine	0.95	0.01	0.73	0.15
testosterone	1.19	0.18	0.64	0.04

**Table 1.** Absorbance was measured in endpoint mode at 280, 300, 320, 340, 360, and 800 nm on a UV vis microplate spectrophotometer (SpectraMax<sup>®</sup> Plus, Molecular Devices). Aqueous solubility of each drug was calculated from the maximum absorbance units (AU) at each wavelength.

**Figure 1.**

**Biomek vs. Manual Screening Method**



**Figure 1.** Data represents the correlation of manual and automated screening methods. Each data point represents the solubility ratio of the drug from one plate per method. Biomek ratio, n=9. Manual ratio, n=6.

## Conclusion:

The MultiScreen Solubility plate provides a high throughput means to estimate the aqueous solubility of hundreds of compounds per day. It offers superior drug recovery for reliable determination of soluble compound concentration, with the benefit of insoluble particle retention to reduce the chance of false positive readings. Using a single point calibration, the screening ratio is simply and quickly derived thus allowing for compound solubility approximation. Multiple samples, each requiring approximately 200 nanomoles (~100 µg) per result, can be run in parallel. The method allows for the analysis of approximately 45 compounds per plate (in duplicate) with the capability of completing six or more plates in a standard 8-hour day. This assay is inherently compatible with the manner in which most compound libraries are produced (e.g. as stock solutions in DMSO) and is easily integrated into existing chemical profiling and early ADME workflows.

## Ordering Information:

MultiScreen Solubility Plate	Part Number	Package Size
	MS SLB PC 10	10

Millipore Vacuum Manifold      MAVM0960R

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