

Multiwell Microfluidic Plates for Evaporation-Controlled Sub-microliter Assays : Design and Results

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Rapid throughput screening relies on instruments and devices that allow simultaneous measurements from multiple reaction reservoirs. The standard biological and pharmaceutical platform is a card of about 127 x 86 mm size, with 96 or 384 assay wells with a standardized (9 mm or 4.5 mm) spacing. A typical measurement on such a card consists of some chemical reaction that results in fluorescence change and such change is then recorded by a commercial fluorescence card reader. Since the well placement is standardized, the readers are typically quite universal. Thus, while retaining the basic geometry and well layout, it is possible to design a titer plate with new properties or functions, but still readable on the existing readers.

Commonly practiced homogeneous assays incubate enzyme, substrate, and a candidate inhibitor for a period typically between 5 and 90 minutes, and then quantify the amount of product created. The more effective the inhibitor is, the less product will be formed. Such assays are typically done in standard 96-well or 384-well plates with relatively large volumes of liquid used. The customary 96-well plates use up to 200 μ L per well, or the 384-well plates use more than ten μ L – which for some assays is considered unsuitable, because the cost of the analyte may be prohibitive, or simply due to the unavailability of enough critical reagent. As the typical screen assay may include hundreds of thousands of test compounds, a complete assay series consumes significant volumes of the expensive or hard-to-obtain enzymes and/or test compounds.

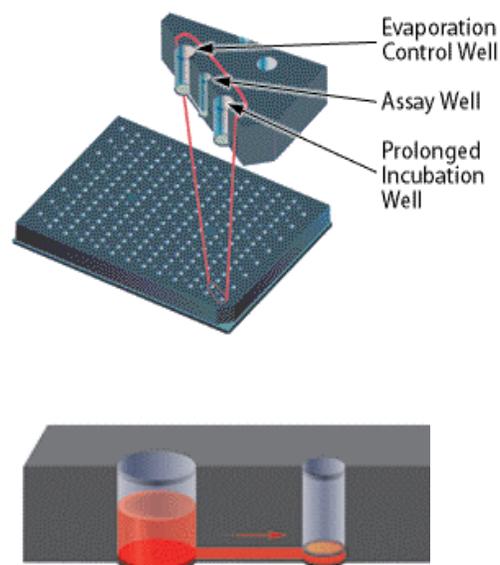
Lowering volume is the clear path to reducing amount of analyte used, but it is not a simple path. Commonly practiced assays incubate enzyme, substrate, and a candidate inhibitor for a period typically between 5 and 90 minutes; the typical obstacle is that with reduced volume, evaporation of the solvent during the course of measurement becomes serious issue that hampers successful use of the small well microtiter plate technology.

The Arteas™ technology, introduced by our company, aims to alleviate the evaporation problems by incorporating in the structure of the assay well a buffer replenishment well, which, during the course of the measurements, maintains constant volume in the assay well while evaporation still naturally proceeds.

The two wells, assay and evaporation control, are connected by a channel through which they communicate. Therefore, measures are taken to limit the amount of assay material that would disappear from the reaction – assay compartment. Computer modeling confirms the practical experimental findings. Mass transport occurs from the large diameter center assay well into the small cross sectional area channels at the well bottom. The

diffusing substance initially enters the channel at a high concentration and forms a “diffusion block”, i.e., substantially decreases the concentration gradient from the well to the channel. Subsequent diffusion rates from the center well to the channel are decreased.

The principle of the operation is described in the following figure:



Top: Drawing of a homogeneous assay microfluidic card with exploded view of the microstructure; **Bottom:** Expanded cross sectional view of evaporation-control (left) and assay (right) reservoirs.

The card-based system consists of 96 pairs of microfluidic reservoirs. Each pair is connected by a microchannel. The microfluidic design and surface chemistry are such that the fluidic height differential in the wells is maintained as shown in the figure, and evaporation from the assay well (typically containing a few hundred nanoliters) is replenished by flow from the evaporation-control well (typically holding several microliters). Assays of 200 nl total volume have been demonstrated, requiring only 10 – 50 nl of precious reagents (e.g., enzymes). Incubations up to one hour have been demonstrated without any sealing or humidification. Results using this approach are comparable in accuracy and coefficients of variance (< 10%) to those from 100 μ L assays in standard 96-well plates.