FlashPlate® File #11

Converting 96-Well Assays to 384-Well FlashPlate Assays

Daniel L. Sissors, Ph.D.
and Sally Casto
PerkinElmer Life Sciences

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Abstract

The use of 384-well microplates is an important step in the process of assay miniaturization for high throughput screening. FlashPlate HTS is a new 384-well microplate incorporating the same technology and principles of operation as PerkinElmer's 96-well version. This paper discusses assay development on FlashPlate HTS, and considerations for converting existing assays based on 96-well generic microplates and 96-well FlashPlate microplates to the FlashPlate HTS platform.

Introduction

The goals of assay miniaturization in high throughput screening are to increase throughput, enhance productivity and reduce costs. Numerous microplate formats have been introduced with more and smaller wells, but the same "footprint," as standard 96-well plates. These include 384-, 864-, 1,536-, and even 3,456-well microplates. These higher-density formats offer numerous potential benefits, including:

- Reduction of reagent usage: smaller volumes are required per well.
- Reduction of plate usage (e.g., one 384-well plate replaces four 96-well plates).
- Reduced manpower requirements: one worker can push a larger number of assays through the system in a given amount of time.
- Increased throughput: less time is spent manipulating plates, whether manually or automatically. In some cases, incubations and reactions may also be quicker in smaller formats.
- Reduced physical space requirements: more assays can be processed in a given workspace; additionally, planned assays require significantly less storage space.

The FlashPlate HTS 384-well microplate is the first practical assay platform for miniaturized high throughput screening. As a homogeneous assay platform, it avoids liquid handling and separation steps that can still be problematic at this physical scale. FlashPlate HTS is not subject to largely unresolved problems associated with bead handling and bead-related signal quenching that affect bead assays in 384-well microplates. And it is available precoated with target proteins of research interest, eliminating the cost and difficulty of establishing plate-coating facilities and procedures within HTS labs.

Product Description

FlashPlate is a line of white polystyrene microplates designed for high-volume, in-plate radiometric assays. The interior of each well is permanently coated with a thin layer of polystyrene-based scintillant which provides a platform for nonseparation assays using a variety of isotopes without the addition of liquid scintillation cocktail. ^{3}H, ^{125}I, ^{14}C, ^{33}P, ^{32}P, ^{35}S, and ^{45}Ca have all been used successfully. Until this time, FlashPlate has been available only in 96-well formats.

FlashPlate HTS is a new 384-well plate incorporating the same scintillant technology and principles of operation as the 96-well version. It is currently available in "basic" form (i.e., with no coating other than the plastic scintillant), in packages of 5 plates (SMP400A) or 20 plates (SMP400), or precoated with Streptavidin (SMP410). Other precoats are available through PerkinElmer Life Sciences' Custom Coating Service.

Equipment Availability

Suitable equipment is available for high-throughput processing of FlashPlate HTS plates. Several manufacturers offer 384-well plate readers, and equipment for liquid handling and dispensing, as shown in Table 1. In some cases, these instruments are identical to, or straightforward adaptations of, existing 96-well equipment; in others, manufacturers have developed new instruments for the task, although the basic principles of operation remain unchanged. Because the footprint of the 384-well FlashPlate HTS is identical to that of standard 96-well plates, no changes have been required in plate handling mechanisms, including standalone robotics and integrated stackers and conveyors.

Table 1

Protocol Comparison Manufacturers of Equipment for 384-Well Microplates

Equipment	wallac	Packard	Dynex	Labsystems	Biotek	BMG	Molecular Dev	Hamilton	Molecular Dev	Tecan	Tiertek	Biochem	Matrix	Tropix	Tomtec	Carl Creative Systems	Skatron
Plate Reading																	
384-Well Colorimetric Reader	●			●	●					●							
384-Well Fluorometer	●	●			●	●				●							
384-Well Luminometer	●	●				●								●			
384-Well Plate Counter (Rad)	●	●															
Liquid Handling/Dispensing		●		●				●		●			●		●	●	
Plate Washers											●				●		●

ref: from a publication by Corning CoStar, 9/97.

Dispensers and plate washers for coating 384-well plates with proteins have become available only recently, and their use is considered a specialty that few HTS labs are currently willing to master. (Few HTS labs have the experience, time, or desire to coat even 96-well plates in large volumes.) Thus, for many laboratories, the availability of precoated FlashPlate HTS plates makes the 384-well format feasible for the first time. PerkinElmer Life Sciences currently supplies protein-coated FlashPlate HTS plates on a custom basis. A streptavidin-coated plate is now available, and will be followed by other stock precoats in the future.

Assay Development and Conversion

If assay development involves in-house plate coating, the same concentrations of protein that are used to coat a 96-well plate are a good starting point for converting to the FlashPlate HTS format, and are likely to be near the optimum in many cases. Concentrations of the coating protein should be titrated to determine optimal coating conditions. Coating protocol development should begin with the same steps and formulations of washes, blocking, solutions, etc., as are used for a 96-well assay. Coating and blocking volumes should generally be in the range of 50 µl to 90 µl per well. Higher volumes may result in liquid being carried over from the bottom of one plate to the top of another if plates are stacked for incubation during the coating process.

Assay conditions that work well on a 96-well platform are a good starting point for optimizing a FlashPlate HTS assay. It is important to examine and test both reagent volumes and concentrations. Optimum per-well reagent volumes are typically one-half to one-tenth the volumes used in 96-well plates. Total volumes should be kept to 60 µl or less.

However, reducing reagent concentrations by the same percentage as the reduction in volumes may not be desirable. When reaction volume in a 384-well plate is one quarter that of a 96-well plate, surface area exposed to sample is just under one-half. Optimal assay sensitivity is likely to be achieved when the reagent concentrations are correlated to the surface area. Adding the same mass of tracer, standards and/or test compounds as are used in the 96-well assay, but in smaller, more concentrated volumes, may compensate for the reduced signal that is expected with one-half the surface area.

A reduction in signal when converting to a 384-well format will not necessarily compromise assay robustness and performance, particularly if the 96-well version has more than adequate signal. For example, an adenylyl cyclase activation assay developed at PerkinElmer Life Sciences on 384-well FlashPlate HTS utilizes one-fourth the volume per well but the same mass of reagents, compared to the 96-well assay. This resulted in a reduced assay signal, but the same normalized assay performance (Figure 1).

Figure 1a

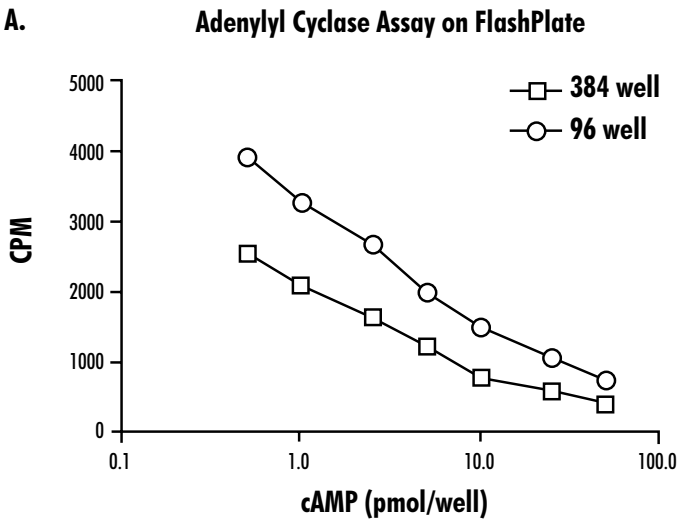
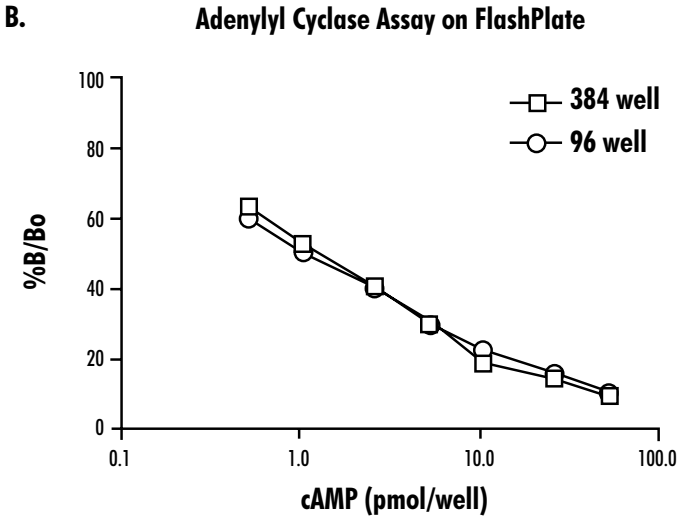


Figure 1b



Cyclic AMP standard curves were run in an Adenylyl Cyclase assay on both 96-well and 384-well antibody-coated FlashPlates. As shown in Figure 1a, the assay signal on the 384-well plate was approximately one-half that of the 96-well plate. However, Figure 1b shows that when the assays are both normalized by %B/Bo, performance is the same. The signal observed in the 384-well version of the assay was still high enough to be considered robust.

When comparing assay performance for an assay converted from a 96-well format to FlashPlate HTS, it is important to examine signal-to-noise ratios as well as raw signal. A 50% reduction in signal is typical for many assays. However, the signal-to-noise ratio may be relatively unchanged. For example, when a FlashPlate 96-well Reverse Transcriptase assay was converted to FlashPlate HTS, the signal-to-noise ratios were nearly equivalent, even though the signal was reduced by half (Figure 2). Generally speaking, as long as a 96-well assay demonstrates sufficient signal-to-noise and repeatability, and the FlashPlate HTS assay produces low backgrounds, reduced signal will not be a problem.

Figure 2a

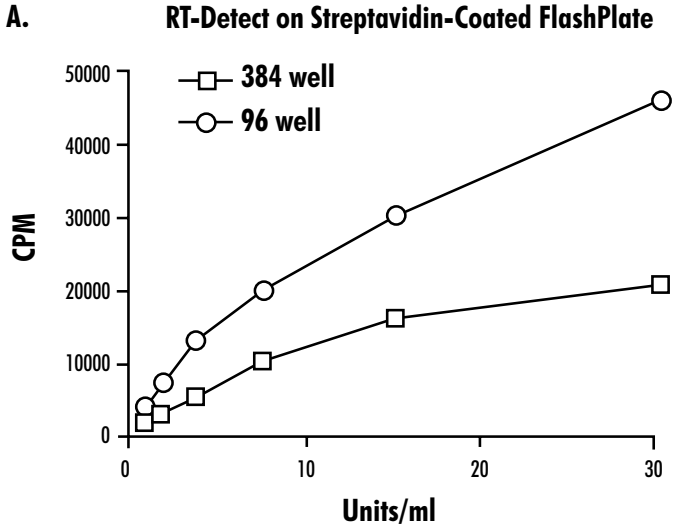
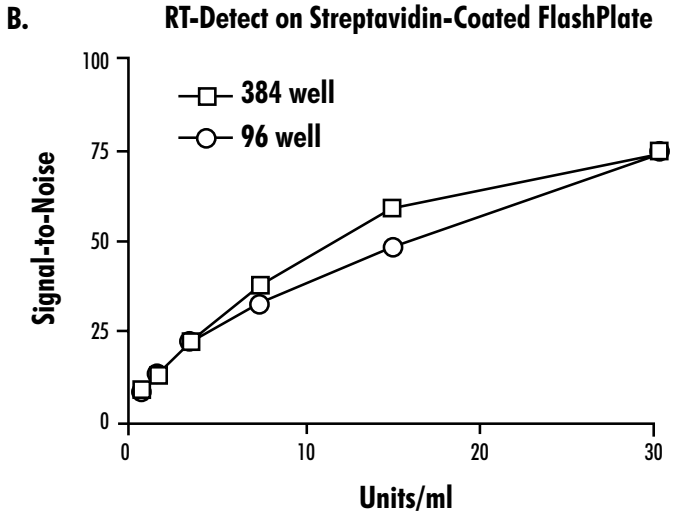


Figure 2b



A Reverse Transcriptase assay was run on both 96-well and 384-well streptavidin-coated FlashPlates. Figure 2a shows that assay signal from the 384-well plate was approximately half that of the 96-well plate. But as shown in Figure 2b, the signal-to-noise ratio was unchanged.

Fluid Dynamics

The physical configuration of the wells of a 384-well plate imposes some special fluid-handling considerations. Because of the very small volumes employed (as small as 5 µl in some cases), some reagents may cling to the side of the well due to surface tension, rather than flow to the bottom. Adding the largest volume last (at least 10 µl) may help wash all the previous additions to the bottom of the well and ensure thorough mixing.

Users of some 384-well microplates have reported problems of well-to-well contamination, caused by "wicking" or capillary action in the corners of the squared wells. Elevated temperatures (37˚ and higher) and extended incubations of 24 to 48 hours further exacerbate this problem. The wells in FlashPlate HTS microplates have corners that are slightly radiused to minimize this problem. Nevertheless, users should keep total well contents at or below 60 µl.

The use of seals should be avoided during intermediate incubation steps, as their removal tends to generate a vacuum (and possibly a static charge) that disturbs well contents, and may cause well-to-well contamination. If it is necessary to cover the plate in order to protect against evaporation and/or contamination, a rigid lid or another plate may be used. The plate should be sealed before counting to avoid contamination of the reader.

Conclusion

FlashPlate HTS is the first homogeneous assay platform optimized for use in a 384-well format. Assays have been developed showing excellent signal-to-noise, and technical requirements for conversion from a 96-well FlashPlate to a 384-well FlashPlate have been shown to be generally straightforward. The 384-well FlashPlate technology can be readily adopted to increase throughput and economy in many high throughput screening applications.

Notes

Worldwide Headquarters: PerkinElmer Life Sciences, 549 Albany Street, Boston, MA 02118-2512 USA (800) 551-2121

Technical Support: in Europe: perkinelmer.europe@perkinelmer.com
in US and Rest of the World: techsupport@perkinelmer.com

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