

Basic FlashPlate®-HTS

SMP400

SMP400E

Product Description

Basic FlashPlate-HTS is a 384-well white plastic scintillator-coated microplate. It is available in packages of 5 plates (SMP400E) or 20 plates (SMP400). It is designed for use with the TopCount® HTS Microplate Scintillation and Luminescence Counter (Packard Instrument Company).

Product Application

FlashPlate-HTS is 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 non-separation assays using a variety of isotopes. It is particularly well suited for binding assays which utilize a protein-coated microplate well to capture a target molecule (e.g., radioimmunoassays). Attempts to miniaturize existing 96-well formats to 384-wells have been impeded by limitations in liquid handling and plate washing devices. Assays performed on FlashPlate typically have less steps and require fewer manipulations than other assay platforms. Thus, FlashPlate-HTS provides a viable platform for high throughput screening assays in 384-well format.

To convert a 96-well microplate assay to a 384-well assay, it is important that both the coating and the assay conditions be optimized for the miniaturized format. The Conversion Guidelines below lists some recommended starting conditions and considerations for adapting a 96-well assay to the FlashPlate-HTS 384-well format.

Conversion Guidelines

I. Coating Plates

Starting conditions for transferring a coating process from a 96-well format to the FlashPlate-HTS 384-well format follow:

- The same concentrations of protein used to coat a 96-well microplate or a bead are likely to be near the optimum in many cases. It is always best to titrate the concentration of coating protein to determine the optimal coating concentration.
- Start with the same plate coating protocol (washes, blocking solutions, etc.) that is currently being used.
- Coating and blocking volumes in the 384 well plates are best in the range of 50 to 90 µL per well. Greater than 90 µL per well may cause plates to stick together if they are stacked during the coating process.

II. Assay Protocol

A starting point for evaluating assay conditions for a FlashPlate-HTS assay is to use the same reagent concentrations, incubation times, etc. as the protocol as for 96-well assay. However:

- Optimal concentrations of some reagents may NOT be the same as for the equivalent 96-well microplate assay. Sensitivity or absolute counts may be improved by adding the same mass of tracer and/or standard/test compounds as used in the 96-well assay, but in a smaller, more concentrated volume.
- Reagent volumes typically are 1/2 to 1/10th the volume used in a 96-well microplate. The total volume in the well should be kept to about 50 to 60 µL or less.
- The order of addition of reagents should also be examined when miniaturizing a microplate assay. Small volumes used in the FlashPlate-HTS wells (e.g. 5 µL) may not flow to the bottom of the well due to surface tension. Adjusting the reagent

volumes such that the last addition is the largest volume ($\geq 10 \mu\text{L}$) can help wash all the additions to the bottom of the well.

- Avoid sealing the plate (use TopSeal, Packard Instrument Company) until you are ready to count the FlashPlate-HTS microplate. Removing the seal between assay steps can cause the contents to be drawn into adjacent wells.
- Use a rigid lid or another plate to cover the assay between steps to reduce evaporation if there is any delay.
- All squared-well 384-well microplates from all manufacturers have a tendency for sample to wick up the corners of the wells due to capillary action. The FlashPlate-HTS design has been optimized to minimize this effect. However, assays at elevated temperatures (37°), extended incubations (more than 24 to 48 hours), assays with large volumes per well (more than about $60 \mu\text{L}$ per well) as well as other conditions may cause some amount of wicking. If your assay must include these conditions you should verify that wicking is not causing the contamination of adjacent wells.

Performance Characteristics

An Adenylyl Cyclase assay was miniaturized from a 96-well FlashPlate format to the 384-well FlashPlate-HTS format as an example miniaturized assay. The following table compares key parameters for these formats.

Parameter	96 Well FlashPlate Format	FlashPlate-HTS (384 Well Format)
Coating volumes <ul style="list-style-type: none"> • Antibodies • Blocking and wash buffers 	100 μL 300 μL	50 μL 70 μL
Coating conditions	Coating conditions (concentrations, buffer formulations, wash and blocking conditions) were identical for the 96 and 384 well formats.	
Concentrations <ul style="list-style-type: none"> • Standards • Tracer 	0.5 to 50 pmol/well 0.18 $\mu\text{Ci/mL}$	0.5 to 50 pmol/well 0.71 $\mu\text{Ci/mL}$ (4X conc. of 96 well assay)
Assay Volumes <ul style="list-style-type: none"> • Standard or cells • Total stimulation volume • Tracer detection mix • Final (total) volume in well 	50 μL 100 μL 100 μL 250 μL	10 μL 25 μL 25 μL 60 μL
Incubation times and conditions	Other assay conditions were identical for the two formats. Plates from each format were run simultaneously for this comparison.	
Performance <ul style="list-style-type: none"> • Curve position ($B_{50\%}$) • Between well (within plate) CV% • Between Plate CV% • B_0 cpm 	1 - 3 pmol/well <10% <10% 6500 cpm	1 - 3 pmol/well <10% <10% 3500 cpm

Although the above represents typical performance for a prototype assay run at NEN, there are many parameters that can be varied when developing an assay and that can affect assay performance. The coating conditions, assay conditions and assay performance that meet your needs may be different from the above example.

Storage

Store FlashPlate microplates at room temperature, protected from light.

FlashPlate is a registered trademark of Packard and is manufactured exclusively for NENTM Life Science Products, Inc. under U.S. patent #4,626,513 and foreign equivalents.