FlashPlate® File #10

The Effects of Solvents on FlashPlate

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Introduction

FlashPlate is a white 96-well polystyrene microplate with plastic scintillant-coated wells. The protein binding characteristics of FlashPlate are similar to other polystyrene microplates, allowing use of standard published plate-coating methods. After a target protein (such as an antibody, receptor, etc.) is bound to the wall of a FlashPlate well, radiolabeled molecules, standards, and samples are added and allowed to bind to equilibrium. The radioactive decay associated with the bound radiolabeled molecules causes a microplate surface scintillation effect detectable on a microplate scintillation counter. Unbound radiolabeled molecules do not activate the scintillant, thus eliminating the need for separation of bound from free.

As FlashPlates are useful for a wide range of applications, a variety of solvents and buffers are used. This article identifies several potential effects of solvents to note when optimizing assays. Adverse solvents may cause the following effects:

1) quenching of the scintillation signal through the introduction of color
2) inactivation of coated molecules or soluble reactants caused by lowered pH
3) removal of coated capture molecules from the FlashPlate surface
4) migration of the scintillant into solution

Assay optimization may require different strategies depending on the application, the solvent used, and its effect. The following observations are offered to facilitate this optimization.

NOTE: These recommendations may not apply to every situation. Each application may have its own unique susceptibilities and must be validated.

Assessment of Effects

Except where noted, solvent-effect test results refer to the binding of [^{3}H]biotin (NET721) to streptavidin-coated FlashPlate (SMP103). The solvent studied is introduced prior to overnight incubation.

Table 1 lists buffers and standard components which have been shown to have no effect (>95% of control) on the binding of [^{3}H]biotin to SMP103. TCA in 5% solution has no effect on [^{3}H]biotin binding to streptavidin. However, in [^{125}I]-cAMP binding to FlashPlate cAMP antibody coated plates (SMP001), 5% TCA inactivates the antibody and destroys the signal.

Table 1

Solvents with No Effect (>95% of control) on [^{3}H]Biotin Binding to Streptavidin-Coated FlashPlate

0.01 M EDTA
0.6 M NaCl
17.6 g/l Sodium Citrate
1% Denhardt's solution (0.1% Ficol, 0.1% PVP, 0.1% BSA)
PBS (10x)
4 N $CsCl_2$
2 M Urea
1% Dextran Sulfate
10% Glycerol
0.1 M Tris pH 7.0
0.1 M Acetate Buffer pH 6.0
1 N HCL
10x Borate Buffer pH 8.2
10 mM $MgCl_2$
1 N LiCl
1% Formaldehyde
0.1 M MOPS
25% DMSO
5% TCA

Table 2 lists conditions that result in a moderate effect (<50% reduction in counts vs. control) on [^{3}H]biotin binding to a streptavidin-coated FlashPlate. (Table 3 lists conditions that result in a large effect (>50% reduction in counts vs. control) on the same testing protocol.) In most cases, the solvent effect may be significantly reduced by reducing the time of exposure, such as using the solvent only in wash solutions rather than during incubation.

Ethanol at 100% extracts the scintillant, but has no effect at 70%. Organic solvents at 100% should be avoided.

Table 2

Solvents with Moderate Effect (5-50% of control) on [^{3}H]Biotin Binding to Streptavidin-Coated FlashPlate

Moderate Effect	
>25%	DMSO
10%	Formamide
2%	Casein
0.1 M	Deoxycholate
0.1%	Triton X-100
0.1%	Tween-20
1%	NP-40
1%	SDS

Table 3

Solvents with Large Effect (>50% of control) on [^{3}H]Biotin Binding to Streptavidin-Coated FlashPlate

Large Effect	
1%	Triton X-100
10%	DMF
100%	EtOH
50-100%	DMSO
1-5 N	NaOH
10%	Formamide / 1.2% Triton X-100
1%	Tween-20 (used in wash)
Flash UV light treatment	

Detergent Effects

Aspiration and washing are unnecessary for most FlashPlate applications. However, to reduce high non-specific binding, washing with detergents may be useful. Studies with [^{125}I]-cAMP binding to cAMP antibody coated FlashPlate (SMP001) indicate that some buffer components may be tolerated in the wash buffer, but not in the overnight incubation. Table 4 lists conditions that result is a small effect (< 25% reduction in counts vs. control) and a large effect (> 50% reduction in counts vs. control).

Table 4

Detergent Effects on cAMP [^{125}I] FlashPlate Assay

	In Incubation	In Wash	Type of Detergent
1% CHAPS	Large effect	No effect	Zwitterion
1% SDS	Large effect	Large effect	Cation
0.5% Triton X-100	Small effect	Small effect	Non-ionic
1% Tween-20	Small effect	Large effect	Non-ionic
1% Cetyl ammonium hydroxide	Large effect	No effect	Anion

Discussion

Adverse solvent effects may be minimized or eliminated by several means, depending upon the application. Every component in the reaction mixture will have its own specific optimal buffer conditions. Any application in FlashPlate must be configured to accommodate each component's limitations, such as pH, salt concentration, DMSO concentration, etc. For quenching of the scintillation signal resulting from colored or cloudy solutions, simple aspiration or aspiration combined with washing will eliminate the problem.

Proteins bind to FlashPlate through hydrophobic interactions. Conditions which denature the protein and alter its hydrophobic regions (e.g., detergents at higher concentrations) will prevent or completely eliminate binding. Assays on FlashPlate should be configured to minimize detergent concentrations or minimize exposure time to the coated FlashPlate.

The most dramatic loss of counting efficiency occurs when the scintillant is extracted from the FlashPlate, or destroyed. This can occur by exposure to 100% organic solutions over periods of several hours or overnight. Sterilizing or cleaning FlashPlate with 70% ethanol does not remove the scintillant. To date, there has been no evidence of scintillant leaching into aqueous solutions or culture media. Even 100% DMSO does not extract the scintillant.

In conclusion, FlashPlate offers a robust platform for the design of non-separation radiometric applications. It resists adverse effects from many solvents. It is flexible enough so that adverse effects produced by other solvents can often be mitigated through modest protocol changes. Thorough understanding of these FlashPlate applications and coating procedures will maximize the success of the user's assay.

For long-term stability, FlashPlates should be stored in their original shipping box or in the dark. Exposure to direct sunlight or high levels of gamma irradiation, electron beam, or UV light will destroy the scintillant. Normal interior light has no effect on FlashPlate performance over the course of a normal assay setup.

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H77913 rev. 01/01