MINIATURIZATION OF SCINTILLATION PROXIMITY ASSAYS TO 384 WELL MICROPLATE FORMAT

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Introduction

Scintillation proximity assay (SPA) is a homogeneous assay technology that has been successfully applied to many drug screening applications. By coupling a range of specific molecules to SPA particles, receptor binding assays, enzyme assays, molecular interaction assays and radioimmunoassays have been established. SPA is applicable to high throughput screening and can be adapted for automation. Assays are routinely performed in 96 well microplates (MP), but many users are converting to utilization of 384 well plates.

The potential advantages of assay miniaturization are as follows:

- reduction in plate usage, as one 384 well plate is equivalent to four 96 well plates.
- reduction in reagent usage, for example 200µl assay can be reduced to 50µl volume
- increased throughput, particularly if the process is automated.

Here we demonstrate SPA assays that have been miniaturized to the 384 well format.

Method

Assay conditions that work in a 96 well platform are a good starting point for optimising 384 well SPA assays. Reagent volumes and concentrations should be optimised for each assay and will be dependent on the assay type. (See Table 1 for examples of assays which have been miniaturized to 384 well format.) There are several different approaches currently available for conversion from 96 to 384 well MPs that can be applied to the miniaturization of SPA assays.

1. Miniaturization using 96 well assay reagent concentrations in a reduced volume.
2. Miniaturization with the same mass of reagents used in 96 well assay.
3. Miniaturization by optimising the reagents used in a 384 well assay.

Assay Name	Product Code	Isotope	Assay volume
Reverse Transcriptase	NK8972	[^{3}H]	50µl
MAP kinase	RPNQ0200	[^{33}P]	75µl
cAMP	TRKQ7140	[^{125}I]	50µl
GTPγS	RPNQ0210	[^{35}S]	50µl
EGF receptor	In house	[^{125}I]	50µl
CHO NK1 receptor	In house	[^{125}I]	50µl
Endothelin receptor	NK8783	[^{125}I]	50µl
Angiotensin II receptor	NK8981	[^{125}I]	70µl
MIP Iα	In house	[^{125}I]	50µl
Sphingomyelinase	TRKQ7140	[^{3}H]	70µl

Table 1. Examples of miniaturized SPA assays.

Approach 1.

To perform assays in a 384 well microplate the total assay volume was reduced from 200µl in a 96 well to 50µl. To use the reagents at the same concentration as the 96 well assay four fold less reagents would be needed i.e. 25% of the 96 well assay reagents were used.

An [^{125}I]EGF receptor binding assay was miniaturized to 384 well using the same concentration of reagents as the 96 well assay . A431 cell membranes were pre-coupled to WGA PVT SPA beads at 50µg/ml and reagents were combined together for dispensing into the 384 well microplates. Figure 1 shows the binding of 400 pM [^{125}I]EGF to A431 cell membranes over 4 hours in both assay formats. Non-specific binding was determined in the presence of 150nM EGF. A signal : noise ratio of 10:1 was observed in both assays and the assay sensitivity was maintained with SPA signals of 20,000cpm and 2,500cpm observed in the 96 and 384 well assays respectively.

The [^{35}S]GTPγS SPA binding assay (RPNQ 0210) used for the quantification of agonist induced activation of G protein coupled receptors has also been miniaturized to 384 well by maintaining the reagent concentrations and reducing the assay volume. The effect of 10µM phenylisopropyladenosine (PIA), a known agonist of the adenosine A1 receptor on [^{35}S]GTPγS binding to cloned A1 receptors expressed in CHO cells, was determined in both 96 and 384 well microplates. Reagents were combined together for dispensing into the 384 well microplate and the reactions were incubated at room temperature for 30 minutes. The plates were centrifuged at 1000g for 10 minutes to reduce any non- proximity effects and were counted in a 96 and 384 well microplate counter. The results, illustrated in Figure 2, show that enhanced [^{35}S]GTPγS binding was observed in the presence of agonist in both assay formats. The SPA signal observed in the 384 well assay was lower than the 96 well format, but the 2:1 signal:noise ratio was maintained.

Approach 2.

The reverse transcriptase enzyme assay SPA system (NK8972) was miniaturized to 384 well by using the same mass of reagents as the 96 well assay. The reagents were prepared at concentrations higher than for the 96 well assay. In the 384 well assay reagents were added in approximately 25% of the 96 well assay volume and were used up to four times more concentrated. The results in Figure 3, show that a linear relationship was observed between the incorporation of [^{3}H]TTP and amount of HIV-1 reverse transcriptase up to 0.2 units enzyme in both 96 and 384 well formats.

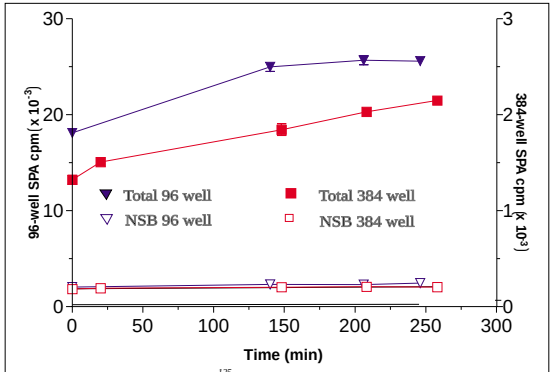

Figure 1. Effect of time on [^{125}I]EGF binding to A431 cell membranes in both 96 and 384 well formats. Values are means ± SEM (n=3).

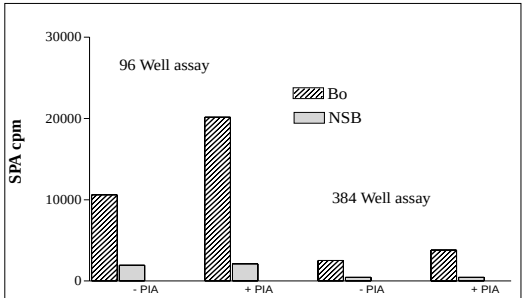

Figure 2. Determination of the effect of 10µM PIA on [^{35}S]GTPγS binding to CHO A1 receptors in both 96 and 384 well microplate assays. Values are means (n=3).

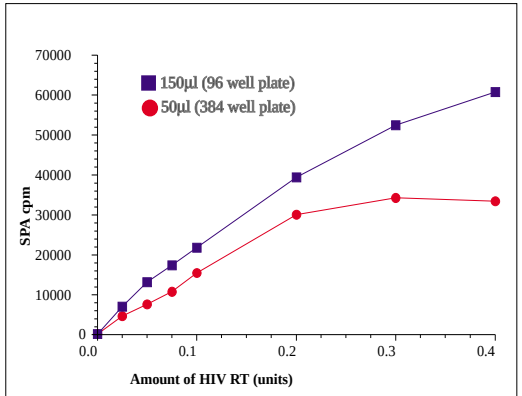

Figure 3. Determination of the effect of varying the amount of HIV-1 reverse transcriptase. The amount of HIV RT used was varied between 0.025 and 0.4 units. Values are means (n=3).

Approach 3.

The amount of reagents used in the [^{33}P] MAP kinase SPA assay system RPNQ0200 have been optimised for performing the assay in a 384 well microplate and to reduce any non-proximity effects. The inhibitory effect of staurosporine on [^{33}P] ATP phosphorylation of a biotinylated myelin basic protein substrate by Erk-1 MAP kinase was determined in both 96 and 384 well microplates using the conditions shown in Table 2. In a 30 minute assay at 37°C without staurosporine present, 25,000 SPA cpm and 7000 SPA cpm were observed for the 96 and 384 well formats respectively, and a signal:noise ratio of 6:1 was maintained. The results illustrated in Figure 4, show that in the presence of staurosporine comparative IC_{50} values of 4.35µM and 4.15µM were observed for 96 and 384 well SPA assays respectively.

	96-well		384-well	
	Vol	Amount / Concentration	Vol	Amount / Concentration
bMBP substrate/ATP	5µl	125pmol/ 50pmol	5µl	50pmol/ 25pmol
[^{33}P]ATP	5µl	0.5µCi	5µl	0.1µCi
Enzyme	10µl	1.0µg	10µl	0.5µg
Assay buffer	20µl	-	5.0µl	-
Staurosporine	10µl	0.001-100µM	10µl	0.001-100µM
SPA beads	200µl	0.5mg	20µl	0.25mg

Table 2. [^{33}P]MAP Kinase SPA assay protocol.

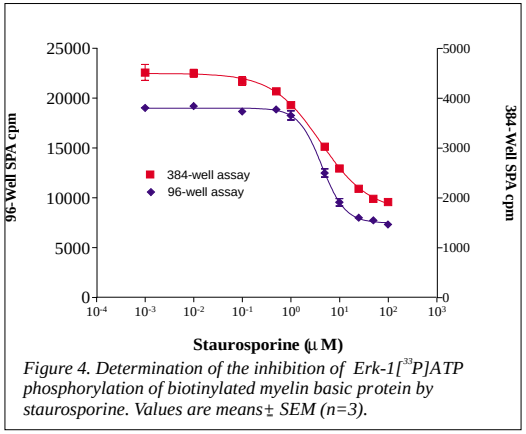

Figure 4. Determination of the inhibition of Erk-1[^{33}P]ATP phosphorylation of biotinylated myelin basic protein by staurosporine. Values are means± SEM (n=3).

Discussion

We have demonstrated different approaches which can be used for miniaturization of SPA assays to 384 well microplates. The assay can be performed at reduced volume, reduced mass or completely re-optimised. The chosen approach will depend on the original 96 well assay performance. The benefits obtained will vary, but in most cases the miniaturized assay will have significant advantages over the 96 well assay with reduced reagent usage and an increase in throughput.

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