Using High Throughput SPA To Screen for CYP2D6 Interactions

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Introduction

Scintillation Proximity Assay (SPA) is an innovative approach to high throughput screening which allows the rapid and sensitive assay of a wide variety of biological processes in a homogeneous system. It is versatile, can be readily automated and assay costs are reduced as the requirement for filters, washing steps and scintillation reagents is eliminated.

The SPA principle is based on the observation that in aqueous solution weakly emitting β-isotopes such as [^{3}H] need to be close to scintillant molecules to produce a light signal. If not, the energy is dissipated into the aqueous solvent.

We have developed a scintillation proximity assay (SPA) using yttrium silicate (YSi) beads, suitable for the measurement of CYP2D6 interactions through the inhibition of [^{3}H]imipramine binding to recombinant CYP2D6 co-expressed with NADPH-cytochrome P450 reductase (CYPOR)[(1)]. Residual binding of the radioactive ligand is determined by scintillation counting following incubation of the test compound with CYP2D6/CYPOR and NADPH (1mM) for up to 30 minutes at 37°C.

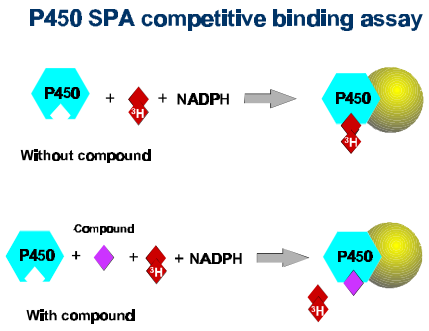

Figure 1. *P450 SPA assay concept.*

Method

Using a 96-well microplate, 10µg CYP2D6 (0.45pmol) in 25µl PBS was pre-incubated with the test compound (final concentration 10µM) at 37°C for 15 minutes.
This was followed by the addition of 0.1µCi [^{3}H]imipramine (Amersham Biosciences, TRK553, 20-30Ci/mmol, ~150-200,000 liquid scintillation counts added) and 1mM NADPH, 25µl each.
The reactions were further incubated for 30 minutes at 37°C. 1mg YSi SPA beads (Amersham Biosciences RPNQ0013) was added to each well, the plate was shaken for 10 minutes and counted using a Wallac MicroBeta™ scintillation counter. Total assay volume was 150µl. Results may be obtained for up to18 hours after the bead addition (see Figure 2).

Results

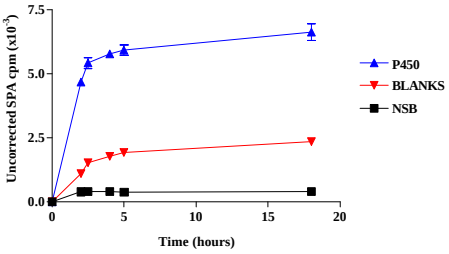

Figure 2. *Typical CYP2D6 assay results in the absence of test compounds, shown as means, +/- SEM, (n=3).*

"Blanks" are microsomes prepared from mock-infected cells, this signal was subtracted from the P450 counts to give corrected specific SPA cpm. NSB (non-specific binding) was calculated from wells containing PBS, [^{3}H]imipramine and NADPH only.

The uncorrected P450 SPA signal generated was typically >4000cpm. Signal:noise, calculated as the P450 SPA signal divided by NSB, was generally > 10:1.

This assay has been successfully used to distinguish a panel of known compounds that bind to CYP2D6 from a series of P450 probe compounds.
The screening results shown as SPA cpm in Table 1 and Figure 3 are from counting the plates immediately, and are means, +/- SEM,(n=3).

ID	COMPOUND	P450	BLANKS	CORR[a]	% OF CONTROL
CONTROL	NO COMPOUND	5023	1100	3923	100
1	BUFURALOL	1921	1139	782	20
2	ONDANSETRON	2501	1152	1349	34
3	AMITRIPTYLINE	1373	1035	338	9
4	DEXTROMETHORPAN	1365	1137	228	6
5	IMIPRAMINE	1329	1098	231	6
6	PROPANALOL	1520	1189	331	8
7	KETOCONAZOLE	3141	1195	1946	50
8	FURAFYLLINE	4045	1322	2723	69
9	PHENACETIN	5602	1252	4350	111
10	CHLORZOXAZONE	4957	1352	3605	92

Table 1 *Results and details of the compounds screened at a final concentration of 10mM.*

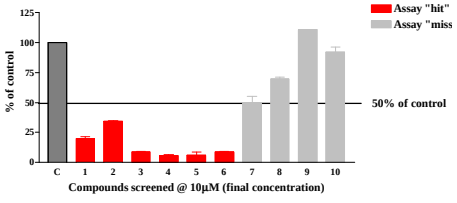

Figure 3 *Typical CYP2D6 screening results.*

From a panel of 10 P450 probe compounds, 6 were correctly identified as having interactions with CYP2D6.
This assay can be used to generate IC_{50} values (as shown in Figure 4 below) with, for example, quinidine, a well known inhibitor of CYP2D6.

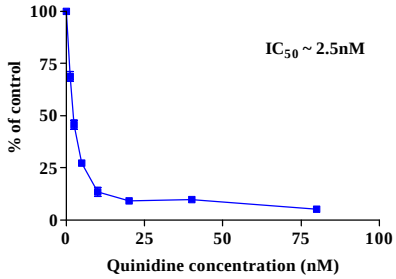

Figure 4 *Typical IC_{50} result achieved with quinidine, a potent inhibitor of CYP2D6.*

CONCLUSIONS

- We have developed an SPA assay for the measurement of CYP2D6 interactions which is suitable for the rapid screening of compounds in microplate format.
- The assay utilises [^{3}H]imipramine and has been successfully used to distinguish known compounds that bind to CYP2D6 from compounds that bind to other CYP isoforms.
- An IC_{50}value of ~2.5nM was generated with quinidine, a well known CYP2D6 inhibitor.

Reference

1. HOOD,S.R. *et al., Methods in Mol.Biol.,* **107,** 203-218, (1998).

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