



# Label-Free High-Throughput Screening by Mass Spectrometry

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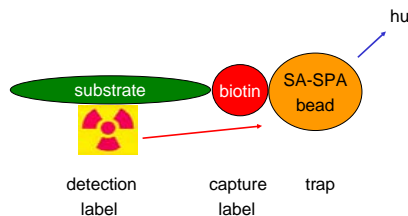
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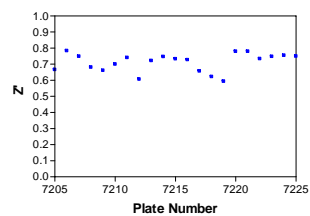
## Abstract

Label-free screening technologies are becoming increasingly important in the drug discovery process, as therapeutic areas such as oncology and cardiovascular disease continue to explore intractable screening targets. Mass spectrometry provides an excellent tool for screening enzymatic targets for several reasons; high specificity and sensitivity, direct detection of substrates and products, and applicability to a broad chemical range of unmodified substrates and products. Traditionally, mass spectrometry has been limited in high throughput screening applications due to low throughput (2-3 minutes/sample). In this study, high-throughput mass spectrometry (HTMS) (6-7 seconds/sample) is investigated as a primary screening tool via direct comparison to a radiometric scintillation proximity assay (SPA) using a small set of Protein Kinase B inhibitors. The rank order of IC<sub>50</sub> values from SPA and HTMS are identical and the strong correlation of IC<sub>50</sub> values validates the use of HTMS for the investigation of kinase targets, and suggest utility for the screening of intractable targets.

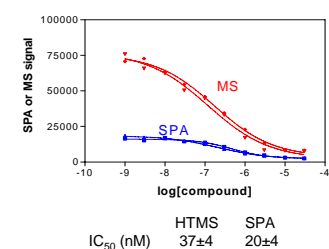
## Scintillation Proximity Assay



## HTMS Assay Performance



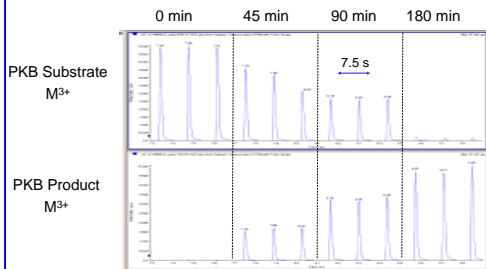
## IC<sub>50</sub> Comparison



## Why HTS by Mass Spectrometry?

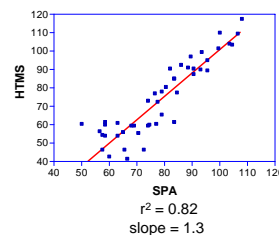
- Mass spectrometry is label free
- overcomes limitation of traditional HTS formats
- Provides assay when a traditional HTS assay is inaccessible
- especially true for non-kinase targets
- Provides an HTS approach to targets that typically require
- fluorescent substrates/probes
- radiolabeled substrates/probes
- coupling assays
- Mass spectrometry can access intractable targets
- portfolio diversity/novelty

## RapidFire™ Mass Spectrometry

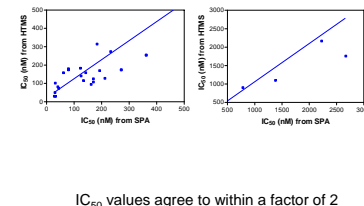


## Correlation of Percent Inhibition

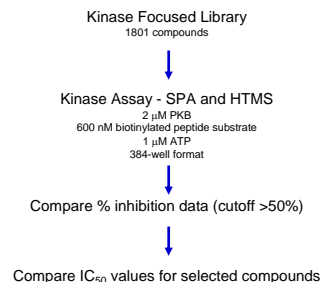
Compounds where percent inhibition >50% for at least 1 well for either SPA and/or HTMS



## IC<sub>50</sub> Comparison

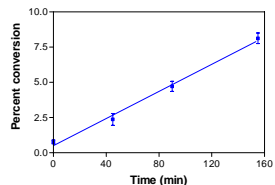


## Study Outline



## RapidFire™ Assay Development

### Linear conversion of peptide substrate



## Percent Inhibition for Individual Wells

Compounds where percent inhibition >50% for either SPA and/or HTMS

Compound	SPA	HTMS	Compound	SPA	HTMS
1	55	61	37	76	72
2	101	98	38	74	63
3	108	109	39	50	51
4	107	108	40	79	80
5	103	104	41	89	74
6	57	73	42	31	63
7	88	89	43	101	99
8	66	54	44	32	32
9	77	77	45	34	34
10	69	69	46	36	36
11	60	57	47	91	88
12	64	61	48	64	49
13	95	92	49	38	38
14	64	63	50	56	59
15	62	64	51	40	40
16	80	78	52	100	91
17	59	58	53	42	42
18	52	48	54	105	104
19	72	64	55	44	44
20	96	96	56	80	75
21	90	78	57	46	46
22	55	86	58	47	47
23	53	53	59	67	64
24	84	83	60	79	62
25	69	59	61	61	56

## Summary

HTMS and SPA applied to a focused library screen of the oncology target Protein Kinase B/AKT.

Good correlation between percent inhibition at a single compound concentration between the two techniques.

Good correlation between IC<sub>50</sub> values obtained by SPA and HTMS.

RapidFire™ HTMS platform is a robust label-free assay format.

Results for the well-characterized kinase PKB provide confidence in HTMS potential for screening intractable targets.