#38

A Comparison of FRET and FP Assay Platforms for a ser/thr Kinase

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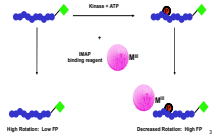
Introduction

In recent years, a variety of non-radioactive assay technologies, such as Fluorescence Polarization (FP) and Fluorescence Resonance Energy Transfer (FRET), have been developed for HTS which offer the promise of increased throughput and cost savings. Two of these formats, the Invitrogen Z'LYTE assay (FRET) and the Molecular Devices IMAP Progressive Binding System assay (FP), were evaluated as assay platforms for a ser/thr kinase. An HTS assay was developed for each format using the appropriate substrate based on the same peptide sequence. The FP assay was selected for the HTS resulting in a robust campaign.

1

FP-based Kinase Assay (Molecular Devices IMAP)



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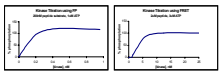
Kinase Titration

The kinase was titrated to determine the optimal enzyme concentration at the limiting peptide condition for each assay format. The ATP concentration was adjusted below it's K_m due to the unsaturating state of the peptide concentration for each assay technology.



The FP assay requires significantly less kinase in order to achieve similar % phosphorylation of the substrate peptide.

5

Comparison of HTS Conditions

	FP	FRET
[peptide], µM	0.200	2
[ATP], µM	1	3
[kinase], nM	0.030	2
% phosphorylation	18	60
Signal window	20.1	11.5
Z'	0.80	0.66
Signal:noise	18.3	12.4
Signal:background	5.8	3.4
Kinase rxn time, min	30	45
Signal development time	30	60
Plates processed in 24 hours	180	120

The FP assay was selected for the HTS campaign based upon reagent consumption and plate processing time.

7

Conclusion

Two fluorescent assay technologies developed for kinases, the Invitrogen Z'LYTE assay (FRET) and the Molecular Devices IMAP Progressive Binding System assay (FP), were evaluated for a ser/thr kinase. Based on this comparison, we draw the following conclusions:

- At an established peptide concentration for each assay technology, a robust FP assay can be run at 18% phosphorylation while the FRET assay must be run at >50% phosphorylation to achieve similar assay statistics.
- The FP assay used ~100 fold less kinase than required by the FRET assay.
- The FP HTS campaign showed consistency across all runs and was able to identify hits with a wide range of potency.

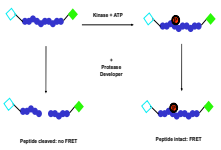
9

Format Comparison

Technology	IMAP	Z'LYTE
Assay Format	FP (non-antibody)	FRET
Assay Type	Signal increasing	Signal decreasing
Detection Mode	Binding the phosphorylated peptide to beads	Phosphorylated peptide maintains FRET signal
Peptide Requirement	Sequence and labeling Limiting in assay to 200nM	Sequence modification and labeling Limiting in assay to 2uM
ATP	<100uM	uM to mM

2

FRET-based Kinase Assay (Invitrogen Z'-LYTE)



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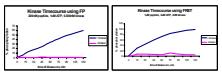
Kinase Timecourse

After determining the ideal enzyme concentration for the substrate conditions of each assay, a timecourse was run to select the appropriate kinase reaction time.

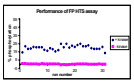


In the FP assay a reaction time of 30 minutes was found to yield a robust HTS assay at 18% phosphorylation. In the FRET assay, a 45 minute kinase reaction time is required to achieve the same robustness and yields 60% phosphorylation.

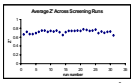
6

Performance of FP HTS

The FP HTS assay performed very well across runs. The average total value was 20% phosphorylation.



The FP assay was extremely consistent across all screening runs. The average Z' value for the HTS campaign was 0.72.



8

Acknowledgements

BMS: Lead Discovery and Profiling

Martyn Banks

Ramesh Padmanabha

Kim Esposito

Lynda Cook

10