Comparison of Kinase Assay Technologies for High Throughput Screening

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1 Abstract

Tyrosine Kinases are important regulators of cellular processes that include cell cycle progression, metabolism, and apoptosis. Kinases have been linked to disease states such as cancer and cardiovascular disease.

PerkinElmer has developed three technologies which can be used to perform tyrosine kinase assays. In this poster, we will describe the performance of the scintillation proximity technology using FlashPlate®, a homogeneous Time Resolved Fluorescence technology using LANCE®, and an Amplified Luminescent Proximity Assay using AlphaScreen®.

Studies were performed to compare sensitivity, precision, and ease of use of each assay. The comparative data shown here will highlight advantages of each technology. These assays can all be easily modified to accommodate many robotic systems and situations in fully homogeneous assays.

2 Introduction

Kinase Technology Comparison

- FlashPlate Kinase Assay
- LANCE Kinase Assay
- AlphaScreen Kinase Assay

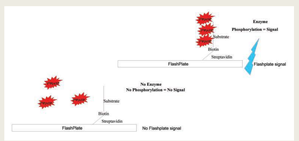
Comparisons

Using Abl enzyme and poly GT substrate

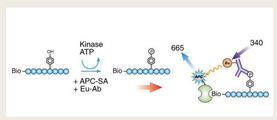
- Enzyme/Substrate Concentrations
- Detection Reagent Needs
- Overall ease of use

3 Basic Principle

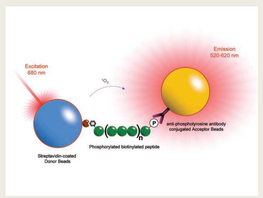
FlashPlate Kinase Assay



LANCE Kinase Assay



AlphaScreen Kinase Assay



4 Reagents

FlashPlate Kinase Assay

- Streptavidin coated FlashPlate (PerkinElmer, Cat # SMP410)
- [33P] ATP (PerkinElmer, Cat # NEG302H)
- Biotinylated Poly GT (PerkinElmer)
- Abl Protein Tyrosine Kinase enzyme (New England Biolabs, Cat # P6050L)
- Abl 10X Buffer (New England Biolabs Cat# B6059S)
- Buffer contains 50mM Tris-HCl, 10mM MgCl2, 1mM EGTA, 2mM dithiotreotol, 0.01% Brij 35, pH 7.5
- EDTA (Gibco Cat # 15575)
- Detection Buffer, 50 mM Tris, 0.5% BSA
- Wash Buffer- Phosphate Buffered Saline (PBS)

LANCE Kinase Assay

- Biotinylated Poly GT
- Abl Protein Tyrosine Kinase enzyme (New England Biolabs, Cat # P6050L)
- Abl Buffer (New England Biolabs Cat # B6059S)
- Buffer contains 50mM Tris-HCl, 10mM MgCl2, 1mM EGTA, 2mM dithiotreotol, 0.01% Brij 35, pH 7.5
- EDTA (Gibco Cat # 15575-038)
- Detection Buffer- 50mM Tris buffered saline with 0.5% BSA
- LANCE Eu-W1024 Labeled P-Tyr-100 antibody (PerkinElmer Cat # ADO161)
- Streptavidin conjugated to Surelight™-Allophycocyanin (PerkinElmer Cat # CR130-100)
- 384-well Optiplate (PerkinElmer Cat # 6007290)

AlphaScreen Kinase Assay (PerkinElmer Cat # 67606200)

- Streptavidin Donor Beads
- Biotinylated Poly GT (PerkinElmer)
- Abl Protein Tyrosine Kinase enzyme (New England Biolabs, Cat # P6050L)
- 10X Abl Buffer (New England Biolabs Cat # B6059S)
- Buffer contains 50mM Tris-HCl, 10mM MgCl2, 1mM EGTA, 2mM dithiotreotol, 0.01% Brij 35, pH 7.5
- P-Tyr 100 Antibody Acceptor Beads
- Detection Buffer of 50mM Tris with 0.5% BSA
- 384 Well White Optiplate (PerkinElmer Cat # 6007290)

5 Assay Protocols

FlashPlate Kinase Assay

- 10 µL [33P] ATP with 10 µM ATP in Abl buffer
- 5 µL Enzyme
- 5 µL Substrate
- Incubate for two hours at room temperature
- 20 µL of EDTA (30mM stock)
- Wash
- Read Plate on PerkinElmer TopCount® NXT Microplate Scintillation and Luminescence Counter

LANCE Kinase Assay

- 10 µL ATP (100 µM in Abl Buffer)
- 5 µL Substrate
- 5 µL Enzyme
- Incubate for two hours at roomtemperature
- 10 µL EDTA (60 mM stock)
- 10 µL SA-APC/Eu-Antibody
- Incubate for one hour at room temperature
- Read on PerkinElmer Victor2V™ Multilabel Reader

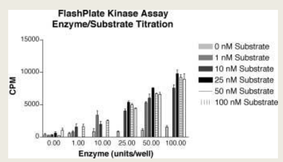
6 Method

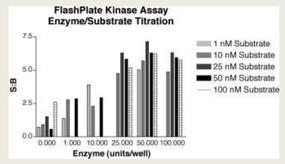
AlphaScreen Kinase Assay

- 10 µL ATP (100 mM in Abl buffer)
- 5 µL Substrate
- 5 µL Enzyme
- Incubate for two hours at room temperature
- 10 µL EDTA (60 mM stock)
- 10 µL Streptavidin Donor Beads
- 10 µL T-Tyr 100 Antibody Acceptor Beads
- Incubate for one hour at room temperature
- Read on PerkinElmer Fusiona™ Multilabel Reader

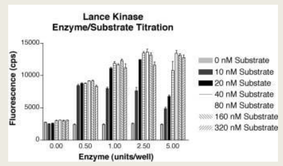
7 Results

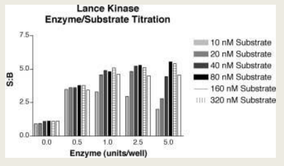
FlashPlate Kinase Assay

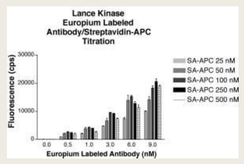




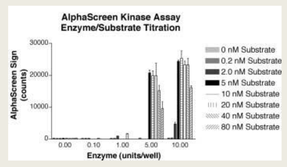
LANCE Kinase Assay

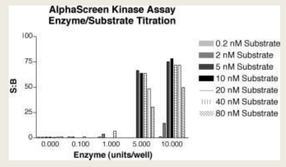






AlphaScreen Kinase Assay





Technology	Optimal Enzyme Units/well	Optimal Substrate Concentration	Signal to Background
Flashplate Kinase	25 Units	25 nM	>6:1
LANCE Kinase	1 Unit	40 nM	>5:1
AlphaScreen Kinase	5 Units	10 nM	>50:1

8 Results (Cont'd)

Technology	Critical Reagents	Amount per well	384-well plate
Streptavidin Flashplate	^{33}P ATP	0.5uCi	192 uCi
	Abl Enzyme	25 Units	9600 Units
	Biotinylated Poly GT	0.5 picomoles	192 picomoles
	ATP	0.1 nanomole	38.4 nanomoles
LANCE	Abl Enzyme	1 Unit	384 Units
	Biotinylated Poly GT	0.8 picomoles	307 picomoles
	ATP	2 µmoles	768 umoles
	Eu-PY 100 Ab	0.12 picomoles	46 picomoles
	Streptavidin APC	0.04 nanomoles	15.36 nanomoles
AlphaScreen	Abl Enzyme	5 Units	1920 Units
	Biotinylated Poly GT	0.2 picomoles	76.8 picomoles
	ATP	2 µmoles	768 µmoles
	SA Donor Beads	0.8 ug	307 µg
	Ab Acceptor Beads	0.8 ug	307 µg

9 Conclusions

Each of the three Kinase Assays presented in this poster are flexible and reliable for use in HTS.

- The Flashplate technology is the gold standard in kinase assays. It is an antibody independent method which makes it a desirable method when no known antibody exists for the kinase of interest. Because it is a solid phase assay, a wash step is commonly added if [33P] ATP is used to improve sensitivity and increase the signal to background ratio.

- The LANCE technology is an antibody based homogeneous technology. It can be further miniaturized to 1536-wells and is readily automated. This makes it an excellent option in the HTS laboratory.

- The AlphaScreen Technology is also homogeneous and easy to automate. Like LANCE it is an antibody dependent technology. It gives the best signal-to-background values of the three assays discussed.

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