

Fluorescent Lifetime Kinase Assays: An Enabling Antibody-Free Platform Technology for Drug Discovery Applications



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Introduction

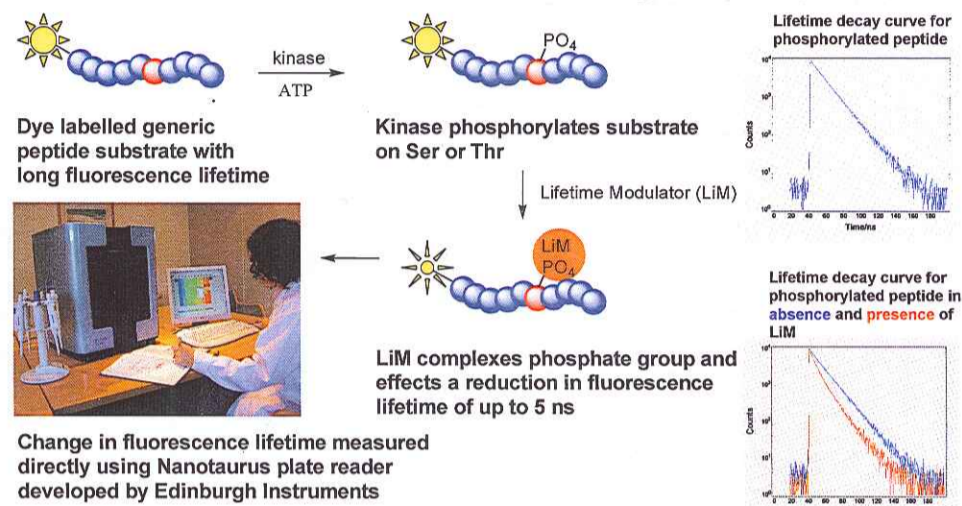
Protein kinases represent one of the most promising groups of drug targets due to their involvement in numerous diseases such as cancer, inflammation, diabetes and neurodegenerative disorders. As a consequence, a number of different biochemical assays have been developed to assess protein kinase activity. However, many suffer from drawbacks associated with radioactive γ -³²P, the requirement for specific antibodies, numerous wash-cycles, and/or interference from fluorescent compounds leading to false positives and negatives.

Fluorescent lifetime (FLT) is defined as the average time a fluorescent molecule spends in the excited state before returning to the ground state. The application of this intrinsic fluorescence property, as the reporting modality in biochemical and cell based assays is attractive, as it is independent of probe concentration and volume, and is unaffected by auto-fluorescence, light scattering and inner filter effects. In addition, FLT enables background interference from fluorescent compound libraries to be minimised, leading to fewer false positives and negatives in drug screening applications

Many commercially available fluorescent dyes have lifetimes of the order of a few nanoseconds, making them unsuitable as reporting systems in FLT based assays. To address this need, Almac Sciences have recently developed fluorescence reporters based on 9-aminoacridine (9AA) possessing lifetimes up to 17 ns. Using these new long lifetime fluorophores, we now report the development of a universal, FLT-based Ser/Thr protein kinase assay platform (**FLEXYTE** protein kinase assays). The assay employs three 9AA-labelled generic substrates (KS 1-3), which together offer coverage for over 100 different kinases, and the use of a lifetime modulator to effect a phosphorylation dependent change in fluorescence lifetime.

This homogeneous, antibody free FLT-based protein kinase assay technology shows excellent promise as a generic, cost-effective, and robust approach for screening this therapeutically important class of proteins.

FLEXYTE Protein Kinase Assay Principle



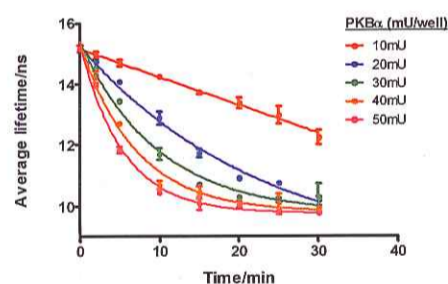
Simple 'Mix and Read' Protocol

- Step 1: Perform kinase reaction with appropriate dye-labelled peptide substrate
- Step 2: Add lifetime modulator and measure fluorescence lifetime directly

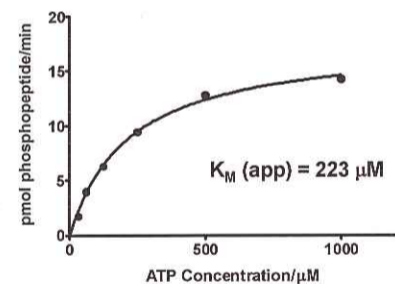
Universal Ser/Thr Protein Kinase Assay

The **FLEXYTE** assay can be quickly optimised for enzyme, substrate and ATP concentration as demonstrated in the panels below for KS-1 with PKB α as the representative kinase.

1. Enzyme Titration



3. ATP Titration

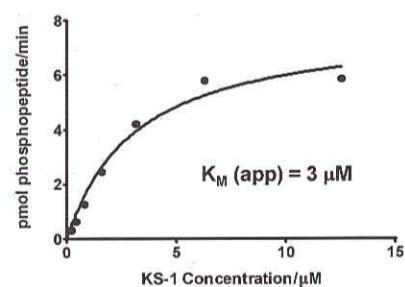


5. FLT vs Radiometric Assays

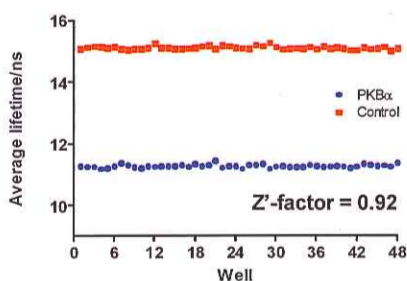
KS-	Kinase	K _M (Sub)/μM		K _M (ATP)/μM		Z'-factor
		Radiometric	FLT	Radiometric	FLT	
1	PKB α	2	3	140	223	0.92
2	PKA	30	2	20	64	0.93
3	CHK1	5	7	50	135	0.93

- Kinetic parameters determined using FLT protein kinase assays are in good agreement to those measured using radiometric methods
- FLT assays gave Z'-factors > 0.9 indicating that they are suitable for HTS applications

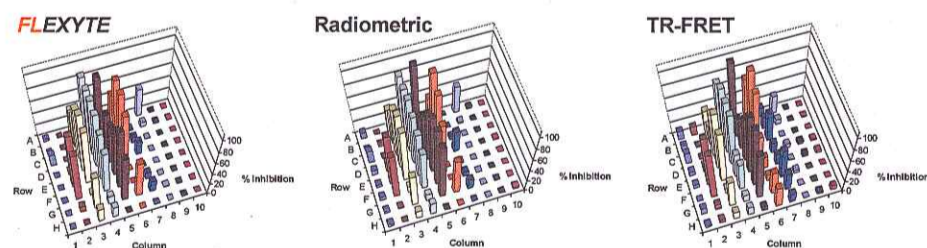
2. Substrate Titration



4. Z'-Factor



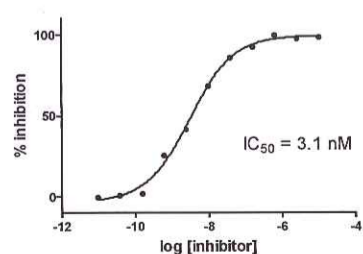
6. Inhibitor Screen



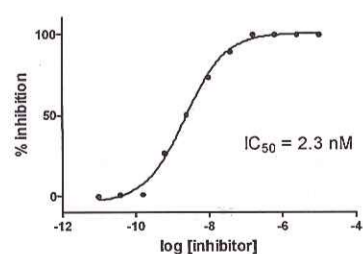
- FLEXYTE** protein kinase assay shows excellent comparison with gold-standard radiometric assay and TR-FRET for hit finding (Pearson correlations: 0.98 and 0.93 respectively)

Staurosporine Inhibition

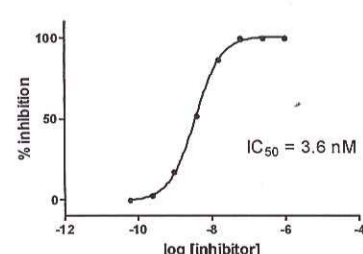
FLEXYTE KS-1: PKB α assay



FLEXYTE KS-2: PKA assay



FLEXYTE KS-3: CHK1 assay



- Three generic **FLEXYTE** peptide substrates enable assays to be configured for >100 Ser/Thr protein kinases
- FLT assays already developed for: PKB α , PKB β , PKB γ , p70s6K, SGK1, MSK1, PKA, ROCK-II, CHK1, MAPKAP2, PRAK1

Conclusion

- A universal **FLEXYTE** protein kinase assay has been developed for Ser/Thr kinases based on fluorescent lifetime as the reporting modality
- This platform technology provides an antibody free, non-radioactive, and cost effective solution for protein kinase screening
- FLT assays have been developed for 11 different Ser/Thr kinases and utility for drug screening applications demonstrated
- Generic substrates enable **FLEXYTE** assays to be configured for a broad panel of Ser/Thr protein kinases in a rapid fashion (covering >100 different kinases)
- FLEXYTE** assay approach applicable to any protein kinase with appropriate substrates
- FLEXYTE** assays have also been developed for proteases and are in development for lipid kinases