

Serine/Threonine Kinase Profiling using the LANCE® Ultra KinaSelect™ Ser/Thr Kit

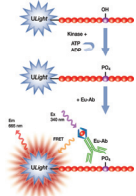
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1 Introduction

Kinases represent one of the largest classes of drug targets actively pursued by pharmaceutical companies. The LANCE® Ultra KinaSelect™ Ser/Thr kit is intended for selecting the optimal peptide substrate for serine and threonine (Ser/Thr) kinase assays. Kinase activity is measured in a LANCE time-resolved fluorescence resonance energy transfer (TR-FRET) assay using five different ULight™-labeled peptide substrates with their corresponding europium (Eu)-labeled anti-phospho-antibodies. ULight is a low molecular weight, red-shifted acceptor dye that replaces the classical APC-streptavidin conjugate. Peptide substrates in the kit include ULight-CREBtide (Ser133), ULight-Myelin Basic Protein, ULight-PLK (Ser137), ULight-Histone H3 (Thr3) and ULight-p70 S6K (Thr389). In this poster, we present data showing the validation of each of the five ULight-peptide/Eu-anti-phospho-peptide antibody pairs against a panel of 185 Ser/Thr kinases.

2 Assay Principle



In LANCE Ultra kinase assays, the phosphorylation of a ULight peptide substrate is detected with a specific anti-phospho-peptide antibody (Ab) labeled with europium chelate molecules (Eu). The binding of the Eu-antibody (donor) to the phosphorylated ULight peptide (acceptor) substrate brings both the donor and acceptor dye molecules into close proximity. Upon irradiation at 320 nm, the excited europium chelate transfers its energy to the nearby ULight dye molecule that will in turn emit light at 665 nm. The intensity of light emission is proportional to the level of the ULight peptide phosphorylation.

3 Assay Method

Step 1: Enzymatic Reaction

+ 5 µL Enzyme (50 nM in kinase reaction)
+ 5 µL ULight-Substrate/ATP or ULight-Substrate
(50 nM/200 µM or 50 nM in kinase reaction)



Step 2: Stop Enzymatic Reaction

+ 5 µL EDTA (10 mM in final volume)



Step 3: Detection

+ 5 µL Eu-anti-phospho-substrate Antibody
(2 nM in final volume)



4 Profiling Data

Table with 9 columns: Supplier, Group/Category, Kinase, Signal to background ratio (Original at 665 nm / control minus ATP at 665 nm), ULight PLK1, ULight MAP, ULight Histone, ULight Myelin, ULight p70 S6K. The table lists 185 kinases and their corresponding signal ratios for each of the five ULight substrates.

* Assay was performed in kinase buffer with 1 mM CaCl₂ and 0.5 µM Calmodulin

** Assay was performed in kinase buffer with 3 mM MnCl₂

5 Kit Components

Reagent	Item Number	Core Motif ¹
ULight-CREBtide (Ser133)	TRF0107-C	RRP $\overline{\text{SY}}\text{RK}$
Eu-anti-phospho-CREBtide (Ser133)	TRF0200-C	
ULight-Myelin Basic Protein Peptide	TRF0109-C	
VTPRIPPP		
Eu-anti-phospho-Myelin Basic Protein	TRF0201-C	
ULight-PLK (Ser137) Peptide	TRF0110-C	RRR $\overline{\text{S}}\text{LLLE}$
Eu-anti-phospho-PLK (Ser137)	TRF0203-C	
ULight-Histone H3 (Thr3/Ser10) Peptide	TRF0125-C	
AR $\overline{\text{T}}\text{KQTA}$		
Eu-anti-phospho-Histone H3 (Thr3)	TRF0211-C	
ULight-p70 S6K (Thr389) Peptide	TRF0126-C	
FLG $\overline{\text{T}}\text{YVAP}$		
Eu-anti-phospho-p70 S6K (Thr389)	TRF0214-C	
LANCE Detection Buffer, 10X, 1.5 mL	CR97-100C	

¹Phosphorylation site is underlined

Additional LANCE Ultra Reagents not included in the kit but working with some of the tested Ser/Thr Kinases

Reagent	Item Number	Core Motif ¹
Eu-anti-phospho-Histone H3 (Ser10)	TRF0210	ARK $\overline{\text{S}}\text{TGG}$
Eu-anti-IkappaB-alpha (Ser32/36) Peptide	TRF0113	RHD $\overline{\text{S}}\text{GLD}\overline{\text{S}}\text{M}$
Eu-anti-phospho-IkappaB-alpha (Ser32/36)	TRF0206	
ULight-mTOR (Ser2448) Peptide	TRF0119	TRT $\overline{\text{D}}\text{SYSAG}$
Eu-anti-phospho-mTOR (Ser2448)	TRF0209	
ULight-Crosstide (GSK-3a Ser21)	TRF0106	
RPRT $\overline{\text{S}}\text{FAEG}$		
Eu-anti-phospho-Crosstide (GSK-3a Ser21)	TRF0202	

¹Phosphorylation site is underlined

6 Results and Conclusions

- Current data demonstrate that 151 of 185 Ser/Thr kinases (82%) phosphorylated at least one of the five ULight peptide substrates included in the kit.
- Nine additional kinases can be targeted with standalone LANCE Ultra products not included in the kit.
- Once one or more ULight-substrates are identified, assay development and optimization can then be completed using larger sizes of standalone reagents of the selected LANCE Ultra product pair.
- As anticipated, many kinases from the MAP kinase pathway do not phosphorylate peptides efficiently. Better results can be obtained with physiological protein substrates or in a cascade assay.
- This new kit allows rapid identification of a suitable peptide substrate for subsequent HTS of Ser/Thr kinases as well as specificity profiling.