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

Julie Blouin, Valérie Paquet, Martina Bielefeld-Sevigny, Jaime Padrós, Nathalie Bouchard, Claire Normand, Liliana Pedro, Marjolaine Roy and Lucille Beaudet*

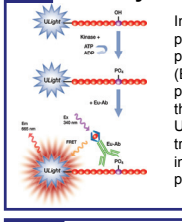
PerkinElmer LAS, Inc. 1744 William St, Suite 600, Montreal, Quebec H3J 1R4, Canada

PerkinElmer precisely.

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 U*Light*™-labeled peptide substrates with their corresponding europium (Eu)-labeled anti-phospho-antibodies. U*Light* is a low molecular weight, red-shifted acceptor dye that replaces the classical APC-streptavidin conjugate. Peptide substrates in the kit include U*Light*-CREBtide (Ser133), U*Light*-Myelin Basic Protein, U*Light*-PLK (Ser137), U*Light*-Histone H3 (Thr3) and U*Light*-p70 S6K (Thr389). In this poster, we present data showing the validation of each of the five U*Light*-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 U*Light* 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 U*Light* 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 U*Light* dye molecule that will in turn emit light at 665 nm. The intensity of light emission is proportional to the level of the U*Light* peptide phosphorylation.

3 Assay Method

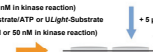
Step 1: Enzymatic Reaction

+ 5 µL Enzyme (20 nM in kinase reaction)
+ 5 µL U*Light*-Substrate/ATP or U*Light*-Substrate (50 nM/200 µM or 50 nM in kinase reaction)

Incubate 1 h at 23ºC with TopSeal-A

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)

Incubate 1 h at 23ºC with TopSeal-A

Read on EnVision (without TopSeal-A)

4 Profiling Data

Supplier	Group/family	Kinase	Signal to background ratio (Signal at 665 nm / control minus ATP at 665 nm): U*Light*-CREBtide	U*Light*-PLK	U*Light*-MBP	U*Light*-Histone H3 (Thr3)	U*Light*-p70S6K
Millipore	AGC / AKT	AKT1PKBα	14.2	4.1	1.0	1.1	1.0
Cama Biosciences	AGC / AKT	AKT2	14.6	12.8	1.0	1.1	1.0
Cama Biosciences	AGC / AKT	AKT3	16.7	20.0	1.0	1.1	1.2
Cama Biosciences	TKL/STKR	ALK3	1.2	1.8	1.0	1.0	1.0
Cama Biosciences	TKL/STKR	ALK4	1.6	1.5	1.0	1.8	1.4
Cama Biosciences	CAMK/CAMKL	AMPKa1	11.0	15.1	0.9	2.3	1.0
Cama Biosciences	Other/AUR	AurA	2.0	14.5	1.1	1.3	1.0
Cama Biosciences	Other/AUR	AurB	13.5	13.3	0.9	2.3	1.2
Cama Biosciences	Other/AUR	AurC	3.9	11.0	1.1	1.1	0.9
Cama Biosciences	AGC / GRK	BARK1	1.1	1.6	1.0	2.5	1.1
Cama Biosciences	AGC / GRK	Bcka/PKB**	1.0	1.1	1.0	1.1	1.0
Cama Biosciences	AGC/GRK	BARK2	1.4	1.6	1.0	10.1	1.1
Cama Biosciences	TKL/RAF	BRAF	2.0	3.0	1.0	9.9	1.1
Cama Biosciences	TKL/RAF	BRAF V600E	1.7	1.9	1.0	9.3	1.1
Cama Biosciences	CAMK / CAMKL	BRSK1	4.4	1.7	1.1	3.4	1.0
Cama Biosciences	CAMK / CAMKL	BRSK2	2.8	1.6	1.0	2.1	1.0
Cama Biosciences	Other/BUB	BUBR1	3.3	1.8	1.0	8.1	2.2
Cama Biosciences	CAMK / CAMK1	CaMKIa*	16.6	1.5	1.0	1.5	1.0
Cama Biosciences	CAMK/CAMK1	CaMKIb*	17.2	1.5	1.0	1.4	0.9
Cama Biosciences	CAMK / CAMK1	CaMKIc*	15.5	1.6	1.0	3.2	2.7
Cama Biosciences	CAMK/CAMK2	CaMKIIa*	14.9	3.2	1.0	7.0	1.8
Cama Biosciences	CAMK / CAMK1	CaMKK*	14.6	6.0	1.0	1.3	1.0
Millipore	CK1 / CK1	Casein Kinase 1	1.2	1.3	N/A	N/A	N/A
Cama Biosciences	CMGC/CDK	CDC2/Cyclin B1	1.2	1.2	43.7	3.6	1.4
Cama Biosciences	Other/CDC7	CDC7/ASK	1.9	1.7	1.0	19.4	1.0
Cama Biosciences	CMGC / CDK	CDK2/CycA	1.0	1.1	39.9	1.5	1.0
Cama Biosciences	CMGC/CDK	CDK2/CycE1	1.4	1.3	38.9	1.6	1.0
Cama Biosciences	CMGC/CDK	CDK4/CycD3	1.4	1.1	37.2	2.0	1.2
Cama Biosciences	CMGC/CDK	CDK5/p35	1.0	1.0	37.1	2.5	1.2
Cama Biosciences	CMGC/CDK	CDK6/CycD3	1.1	1.1	35.7	3.7	5.9
Cama Biosciences	CMGC/CDK	CDK7/CycH/MAT1	1.0	1.0	1.0	1.6	1.1
Cama Biosciences	CMGC/CDK	CDK9/CycC	1.0	1.2	1.0	2.4	1.0
Cama Biosciences	CMGC/CDK	CDK9/CycT1	1.7	1.1	1.0	2.8	1.0
Cama Biosciences	AGC/PKG	CGK2	11.9	12.2	1.1	6.7	1.0
Millipore	CAMK / CAMKL	CHK1	14.6	14.6	1.1	5.5	1.0
Cama Biosciences	CAMK / RAD53	CHK2	14.1	7.3	1.1	4.9	1.0
Cama Biosciences	CK1/CK1	CK1α	1.3	1.6	1.0	4.6	1.0
Cama Biosciences	CK1/CK1	CK1δ	1.0	1.1	1.0	4.8	1.5
Cama Biosciences	CK1/CK1	CK1ε	1.7	1.1	1.0	10.2	1.0
Cama Biosciences	Other/CK2	CK2α1	1.0	1.1	1.0	4.1	1.0
Cama Biosciences	Other/CK2	CK2α2	1.7	1.1	1.0	4.4	1.1
Cama Biosciences	CMGC / CLK	CLK1	4.6	2.0	41.0	2.7	1.0
Cama Biosciences	CMGC / CLK	CLK3	8.0	1.3	11.0	1.5	0.9
Cama Biosciences	STE / STE-Unique	COK**	1.3	1.8	1.0	1.6	0.0
Cama Biosciences	AGC / DMPK	CRIK	U*Light*-Histone H3 (Ser10): S/B ratio = 8.3				
Millipore	CAMK / CAMKL	CTAK1	1.0	1.0	1.0	1.0	1.0
Cama Biosciences	CAMK / DAPK	DAPK1	6.5	18.1	1.0	3.7	0.9
Cama Biosciences	CAMK / DCAMKL	DCAMKL1	7.2	1.7	0.9	2.7	0.9
Cama Biosciences	CAMK / DCAMKL	DCAMKL2	9.3	2.0	0.9	2.5	1.3
Cama Biosciences	TKL/MLK	DLK	14.0	4.0	2.4	1.1	0.9
Cama Biosciences	AGC / DMPK	DMPK1	1.7	1.5	1.0	2.5	1.0
Cama Biosciences	CMGC / ERK5	ERK5	2.0	1.7	1.0	12.6	1.0
Cama Biosciences	Atypical / Alpha	EEF2K*	1.1	1.1	1.1	1.3	1.9
Millipore	CMGC / MAPK	Erk1	1.1	1.0	40.3	1.3	1.1
Millipore	CMGC / MAPK	Erk2	1.0	1.0	39.8	1.0	1.0
Cama Biosciences	CMGC/MAPK	Erk5	1.1	1.0	5.5	1.2	1.0
Cama Biosciences	CMGC/GSK	GSK3β	1.0	1.3	4.6	2.1	1.5
Cama Biosciences	STE/STE20	HGK	2.4	1.6	1.1	6.3	23.8
Cama Biosciences	CMGC / DYRK	HIPK2	1.7	1.5	1.1	1.9	0.5
Cama Biosciences	Other / IRK	IRK6	U*Light* kinase: S/B ratio = 24.0				
Millipore	Other / IRK	IRK2	U*Light* kinase: S/B ratio = 26.6				
Cama Biosciences	Other / IRK	IRK4	4.9	14.2	0.9	4.9	20.0
Cama Biosciences	TKL/IRAK	IRAK1	1.8	2.1	1.1	11.4	26.1
Cama Biosciences	TKL / IRAK	IRAK4*	1.3	1.1	1.5	8.3	32.4
Cama Biosciences	CMGC/MAPK	JNK1	0.9	1.0	1.0	2.7	0.9
Cama Biosciences	CMGC/MAPK	JNK2	0.9	1.0	20.6	0.9	1.0
Cama Biosciences	CMGC/MAPK	JNK3	1.0	1.0	3.9	1.4	1.1
Cama Biosciences	TKL/LISK	LIMK1	1.2	1.0	0.9	1.2	1.0
Cama Biosciences	TKL/LISK	LIMK2	2.8	1.2	1.0	2.4	1.0
Cama Biosciences	CAMK / CAMKL	LKB1	1.9	1.7	1.1	2.7	1.5
Cama Biosciences	CAMK / CAMKL	LKB1*	1.6	1.1	1.0	2.2	1.2
Cama Biosciences	STE/STE20	LOK	1.6	1.1	0.9	1.1	0.9
Cama Biosciences	TKL/LRRK	LRRK2	15.4	13.9	1.1	10.4	1.4
Cama Biosciences	STE / STE7	MAP2K1	1.0	1.1	1.1	1.2	2.5
Cama Biosciences	STE/STE7	MAP2K2	1.5	1.2	1.1	1.6	2.3
Cama Biosciences	STE/STE7	MAP2K3	1.0	1.0	1.0	1.6	1.0
Cama Biosciences	STE/STE7	MAP2K4	1.1	1.1	1.1	1.5	1.0
Cama Biosciences	STE/STE7	MAP2K5	1.3	1.2	1.0	1.6	1.0
Cama Biosciences	STE/STE7	MAP2K6	1.0	1.0	1.0	1.5	1.3
Cama Biosciences	STE/STE7	MAP2K7	1.1	1.1	1.1	1.8	1.0
Cama Biosciences	STE/STE-Unique	MAP3K1α	2.2	3.0	1.0	10.3	1.0
Cama Biosciences	STE / STE11	MAP3K2	1.2	1.1	1.0	1.2	1.7
Cama Biosciences	STE/STE11	MAP3K3	1.3	1.3	1.0	6.2	1.8
Cama Biosciences	STE/STE11	MAP3K4	1.2	1.8	1.0	3.4	1.0
Cama Biosciences	STE/STE11	MAP3K5	1.1	1.1	1.0	3.5	3.8
Cama Biosciences	STE / STE20	MAP4K2	1.1	1.1	1.1	3.6	1.1
Cama Biosciences	CAMK/MAPKAPK	MAPKAPK2	10.3	4.1	37.0	3.4	1.1
Cama Biosciences	CAMK / MAPKAPK	MAPKAPK3	8.2	6.5	1.1	1.5	1.3
Cama Biosciences	CAMK / MAPKAPK	MAPKAPK5	2.3	1.2	1.0	2.8	1.0
Cama Biosciences	CAMK / CAMKL	MARK1	2.8	1.2	1.0	4.8	1.0
Cama Biosciences	CAMK/CAMKL	MARK2	2.5	1.4	0.9	1.6	1.0
Cama Biosciences	CAMK/CAMKL	MARK3	3.3	1.2	1.1	3.9	1.1
Cama Biosciences	CAMK/CAMKL	MARK4	4.6	1.6	1.0	2.3	1.1
Millipore	MAP3K/STE7	MEK1	1.0	1.1	1.4	1.1	3.2

Supplier	Group/family	Kinase	Signal to background ratio (Signal at 665 nm / control minus ATP at 665 nm): U*Light*-CREBtide	U*Light*-PLK	U*Light*-MBP	U*Light*-Histone H3 (Thr3)	U*Light*-p70S6K
Cama Biosciences	CAMK / CAMKL	MELK	13.9	3.0	1.0	1.1	1.1
Cama Biosciences	CAMK/CAMKL	MGC42105	7.2	13.8	1.0	6.6	1.0
Cama Biosciences	STE/STE20	MINK	2.2	1.5	1.0	6.5	54.0
Millipore	MAPK / ERK7	MKK6/SKK3	1.2	1.0	1.0	0.9	1.1
Cama Biosciences	TKL/MLK	MLK1	1.4	1.2	1.2	2.3	4.2
Cama Biosciences	TKL/MLK	MLK2	1.5	1.2	1.0	3.1	2.0
Cama Biosciences	TKL / MLK	MLK3	U*Light*-mTOR: S/B ratio = 4.2				
Cama Biosciences	CAMK / MAPKAPK	MNK1	10.4	2.0	1.0	1.2	1.0
Cama Biosciences	CAMK / MAPKAPK	MNK2	14.9	3.8	1.0	1.6	1.0
Cama Biosciences	Other/MOS	MOS	2.1	1.4	1.0	3.2	1.0
Cama Biosciences	AGC / DMPK	MRCKα	U*Light* Histone H3 (Ser10): S/B ratio = 7.5				
Millipore	AGC / RSK	MSK1	14.6	19.0	3.3	0.9	1.0
Cama Biosciences	AGC / RSK	MSK2	18.2	19.8	2.3	2.3	0.9
Millipore	STE / STE20	MST1	2.7	1.5	1.1	0.0	20.9
Cama Biosciences	STE / STE20	MST2	1.3	1.5	1.1	6.0	4.4
Cama Biosciences	STE/STE20	MST3	4.7	1.2	1.0	1.3	3.1
Cama Biosciences	STE/STE20	MST4	2.8	1.5	1.0	1.4	2.4
Millipore	PIKK/PIAP	mTOR**	1.6	1.4	1.5	2.2	18.3
Cama Biosciences	Other/WEE	MYT1	4.6	2.6	1.0	4.0	1.3
Cama Biosciences	AGC/NDR	NDR1	U*Light* Histone H3 (Ser10): S/B ratio = 5.7				
Cama Biosciences	Other / NEK	NEK1	8.6	1.7	1.3	7.8	56.7
Cama Biosciences	Other / NEK	NEK2	14.5	16.6	1.1	1.8	46.7
Cama Biosciences	Other / NEK	NEK6	1.3	2.3	1.0	2.9	32.0
Cama Biosciences	Other / NEK	NEK7	1.1	1.3	1.0	1.6	33.4
Cama Biosciences	CMGC / MAPK	NLK	1.6	1.3	1.8	1.3	1.1
Cama Biosciences	CAMK/CAMKL	Nuak1	1.1	1.8	1.1	2.0	1.1
Millipore	CMGC / MAPK	p38α	1.9	1.4	16.9	1.0	1.0
Cama Biosciences	CMGC/MAPK	p38β	1.1	1.0	40.6	1.1	1.0
Cama Biosciences	CMGC/MAPK	p38γ	1.0	1.0	33.5	1.5	1.0
Cama Biosciences	CMGC/MAPK	p38δ	1.0	1.0	41.2	1.3	1.0
Millipore	AGC / RSK	p70S6K(T412E)	U*Light* mTOR: S/B ratio = 11.6				
Cama Biosciences	AGC/RSK	p70S6K2	11.1	3.1	1.0	2.0	1.0
Cama Biosciences	STE/STE20	PAK1	1.4	12.3	0.9	2.5	0.9
Millipore	STE / STE20	PAK2	8.3	14.7	1.0	0.9	1.0
Cama Biosciences	STE / STE20	PAK4	2.8	2.0	1.0	1.0	1.0
Cama Biosciences	STE / STE20	PAK6	1.7	2.8	1.0	2.4	0.9
Cama Biosciences	CAMK / CAMKL	PASK	14.2	3.3	1.0	5.4	1.6
Cama Biosciences	Other/TOPK	PBK	1.6	1.6	1.2	1.9	1.6
Cama Biosciences	CMGC/CDK	PCTAIRE1	1.3	1.1	1.1	1.9	1.0
Cama Biosciences	AGC/PKB	PDK1	1.3	1.4	1.0	3.1	1.4
Cama Biosciences	Other/PDK	PDK	0.9	1.0	1.0	10.0	41.6
Cama Biosciences	AGC/PKG	PGK	13.6	10.7	1.0	11.3	1.1
Cama Biosciences	CAMK/PHK	PHKG1	13.0	3.0	1.0	5.5	1.0
Cama Biosciences	CAMK / PHK	PHKG2	14.1	3.3	1.0	6.0	1.5
Cama Biosciences	CAMK / PIM	PIM1	1.8	2.1	1.2	2.4	1.0
Cama Biosciences	CAMK / PIM	PIM2	13.3	2.5	0.9	1.8	0.9
Cama Biosciences	AGC/PKA	PKACα	22.7	11.8	1.0	1.0	1.0
Cama Biosciences	AGC/PKC	PKCα	11.4	10.9	0.9	3.0	1.0
Cama Biosciences	AGC / PKC	PKCβ1	14.5	2.4	0.9	6.3	1.0
Cama Biosciences	AGC / PKC	PKCβ2	13.7	2.4	0.9	3.8	1.0
Cama Biosciences	AGC / PKC	PKCδ	15.3	2.4	1.0	4.2	1.0
Cama Biosciences	AGC / PKC	PKCε	15.7	1.7	1.0	2.9	1.0
Cama Biosciences	AGC / PKC	PKCγ	15.4	4.7	1.0	4.4	1.0
Cama Biosciences	AGC / PKC	PKCη	12.2	2.9	0.9	2.7	1.0
Cama Biosciences	AGC / PKC	PKCι	17.0	2.6	1.0	2.2	1.0
Cama Biosciences	AGC / PKC	PKCθ	15.3	2.8	1.0	3.5	1.1
Cama Biosciences	AGC / PKC	PKCζ	17.1	2.4	0.9	4.2	1.0
Cama Biosciences	CAMK/PKC	PKD1	8.0	24.4	1.0	4.4	1.1
Cama Biosciences	CAMK / PKD	PKD2	14.1	10.3	0.9	1.9	1.0
Cama Biosciences	CAMK/PKD	PKD3	14.4	6.0	1.0	8.8	1.1
Millipore	AGC/PKG	PKGIα	14.0	15.8	1.1	2.0	1.0
Cama Biosciences	AGC/PKN	PKN1	14.1	2.1	0.9	1.6	1.0
Cama Biosciences	AGC/PKN	PKN2	11.9	1.1	0.9	2.3	0.9
Cama Biosciences	Other/PKR	PKR	2.3	1.6	1.0	0.9	1.1
Cama Biosciences	Other / PLK	PLK1	1.8	1.8	1.0	10.4	24.8
Cama Biosciences	Other / PLK	PLK3	4.9	4.0	1.0	6.4	3.9
Cama Biosciences	Other/PLK	PLK4	U*Light* Crosstide: S/B ratio = 4.8				
Cama Biosciences	Other / PLK	PLK4	3.9	1.9	1.0	3.8	1.6
Cama Biosciences	Other / PLK	PRK1	14.9	10.8	0.9	2.7	1.0
Cama Biosciences	TKL / RAF	RAF1	1.6	2.8	1.0	5.5	1.2
Cama Biosciences	TKL/RIPK	RIPK2	2.2	1.4	0.9	9.5	1.5
Cama Biosciences	AGC / DMPK	ROCK1	11.7	10.4	1.0	4.2	1.1
Millipore	AGC/DMPK	ROCK2	16.3	18.0	1.1	1.6	1.1
Millipore	AGC / RSK	RSK1	13.4	2.7	3.8	1.3	1.0
Cama Biosciences	AGC/RSK	RSK2	15.4	15.6	0.8	5.6	0.9
Cama Biosciences	AGC/RSK	RSK3	16.2	18.5	1.0	10.0	1.3
Cama Biosciences	AGC / SGK	SGK	14.3	20.3	0.9	2.7	1.0
Cama Biosciences	AGC / SGK	SGK2	13.7	21.0	1.0	1.4	1.0
Cama Biosciences	AGC / SGK	SGK3	23.3	18.5	1.0	3.1	1.0
Cama Biosciences	CAMK / MLCK	smMLCK	2.0	1.3	1.0	3.3	0.9
Cama Biosciences	CAMK / MLCK	skMLCK	2.2	1.5	1.0	2.7	0.9
Cama Biosciences	CMGC / SRPK	SRPK1	1.0	1.0	0.9	1.0	0.8
Cama Biosciences	CMGC / SRPK	SRPK2	1.0	0.9	1.0	0.9	0.9
Cama Biosciences	STE/STE20	TAOK2	2.7	1.5	1.0	5.0	16.9
Cama Biosciences	TKL / STKR	TGFβR1	1.0	1.4	1.0	1.4	1.0
Cama Biosciences	CAMK/TSSK	TSSK1	3.6	17.9	1.0	5.2	1.0
Cama Biosciences	CAMK / TSSK	TSSK2	11.9	25.2	1.0	3.2	1.0
Cama Biosciences	Other/TTK	TTK	2.3	3.0	1.0	9.3	1.4
Cama Biosciences	CK1 / VRK	VRK1	1.1	1.3	1.0	1.2	1.1
Cama Biosciences	Other / WEE	WEE1	1.5	1.7	1.0	4.4	0.9
Cama Biosciences	Other/WNK	WNK1	1.5	1.5	0.8	1.9	0.9
Cama Biosciences	Other/WNK	WNK2	2.6	1.9	0.9	3.3	1.0
Cama Biosciences	Other/WNK	WNK3**	U*Light* Histone H3 (Ser10): S/B ratio = 7.3				
Cama Biosciences	Other/WNK	WNK4	1.6	1.4	0.9	3.4	0.9

* Assay was performed in kinase buffer with 1 mM $CaCl_2$ and 0.5 µM Calmodulin

** Assay was performed in kinase buffer with 3 mM $MnCl_2$

5 Kit Components

Reagent	Item Number	Core Motif[1]
U*Light*-CREBtide (Ser133)	TRF0107-C	RRPSYRK
Eu-anti-phospho-CREBtide (Ser133)	TRF0200-C	
U*Light*-Myelin Basic Protein Peptide	TRF0109-C	VTPRTPPP
Eu-anti-phospho-Myelin Basic Protein	TRF0201-C	
U*Light*-PLK (Ser137) Peptide	TRF0110-C	RRRSLLE
Eu-anti-phospho-PLK (Ser137)	TRF0203-C	
U*Light*-Histone H3 (Thr3/Ser10) Peptide	TRF0125-C	ARTKQTA
Eu-anti-phospho-Histone H3 (Thr3)	TRF0211-C	
U*Light*-p70 S6K (Thr389) Peptide	TRF0126-C	FLGFTYVAP
Eu-anti-phospho-p70 S6K (Thr389)	TRF0214-C	
LANCE Detection Buffer, 10X, 1.5 mL	CR97-100C	

[1]Phosphorylation 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	ARKSTGG
U*Light*-IkappaB-alpha (Ser32/36) Peptide	TRF0113	RHDSGLDSM
Eu-anti-phospho-IkappaB-alpha (Ser32/36)	TRF0206	
U*Light*-mTOR (Ser2448) Peptide	TRF0119	TRTDSYSAG
Eu-anti-phospho-mTOR (Ser2448)	TRF0209	
U*Light*-Crosstide (GSK-3α Ser21)	TRF0106	RPRTSSFAEG
Eu-anti-phospho- Crosstide (GSK-3α Ser21)	TRF0202	

[1]Phosphorylation 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 U*Light* 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 U*Light*-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.