

Enzalutamide and Apalutamide: In Vitro Chemical Reactivity Studies and Activity in a Mouse Drug Allergy Model

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Table of Contents

Supplemental Data

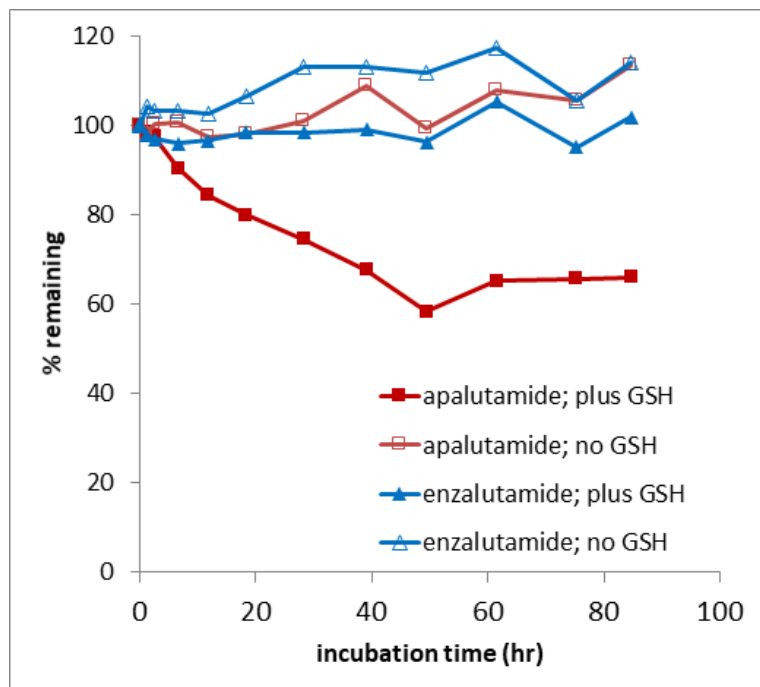
Supplemental Figure 1. Timecourse of apalutamide reaction with GSH	2
Supplemental Figure 2. The 1H-1H COSY Spectrum of the m/z 583 isolate.....	3
Supplemental Figure 3. The 1H-13C multiplicity edited HSQC of the m/z 583 isolate	4
Supplemental Figure 4. The 1H-1H COSY spectrum of the m/z 639 isolate	5
Supplemental Figure 5. The 1H-13C multiplicity edited HSQC of the m/z 639 isolate	6
Supplemental Figure 6. The 1H-13C HMBC spectrum of the m/z 639 isolate.....	7

Supplemental Information

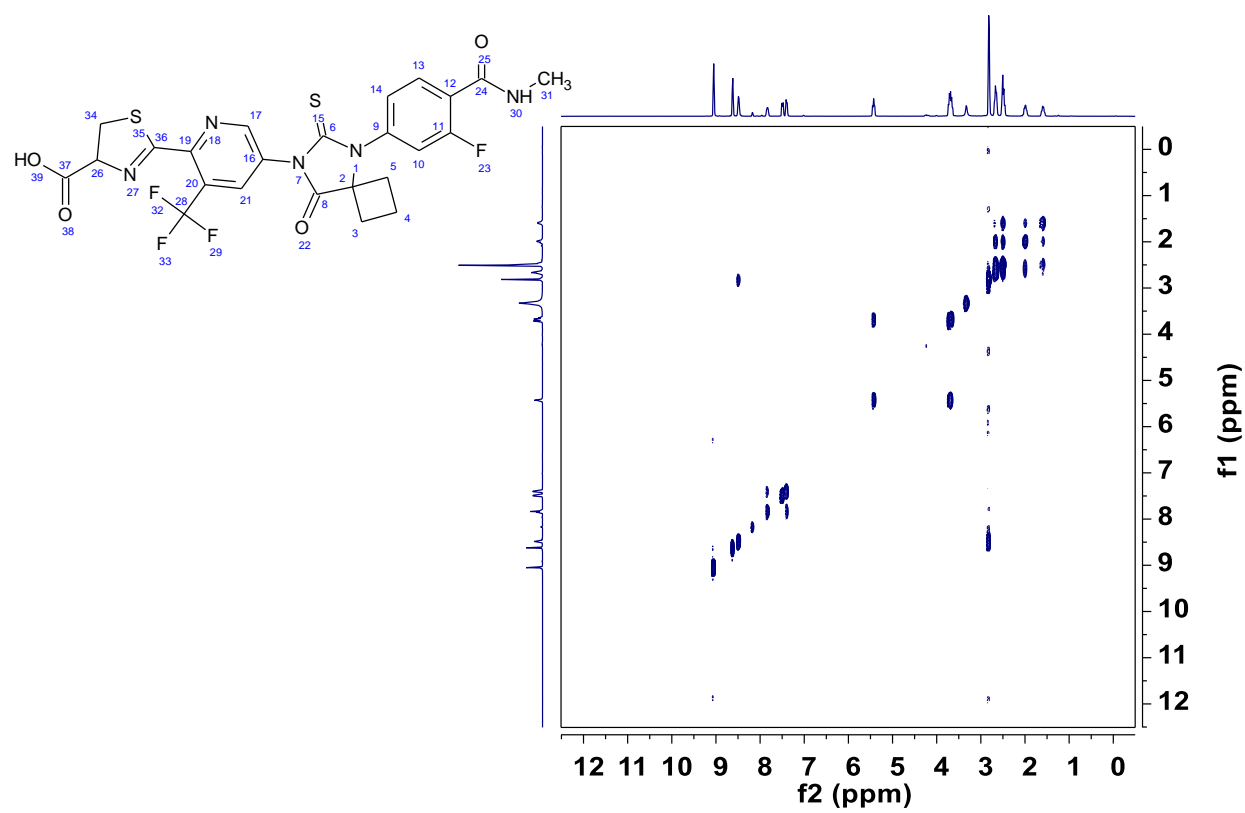
CEREP assay references	8
Thermo Fisher Z'-LYTE assay references	9

Supplemental Data

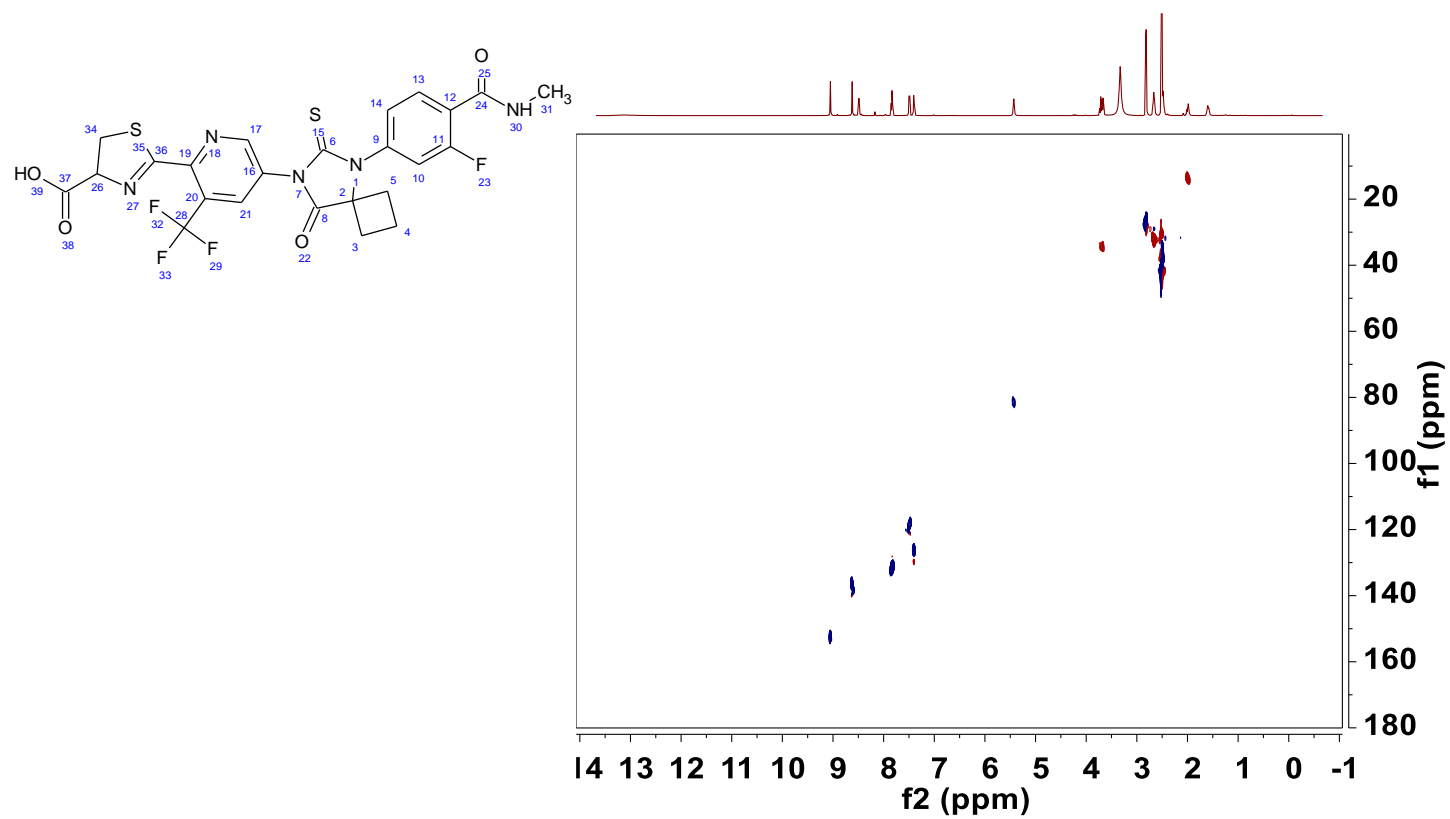
Supplemental Figure 1. Timecourse of apalutamide reaction with GSH. GSH, glutathione.



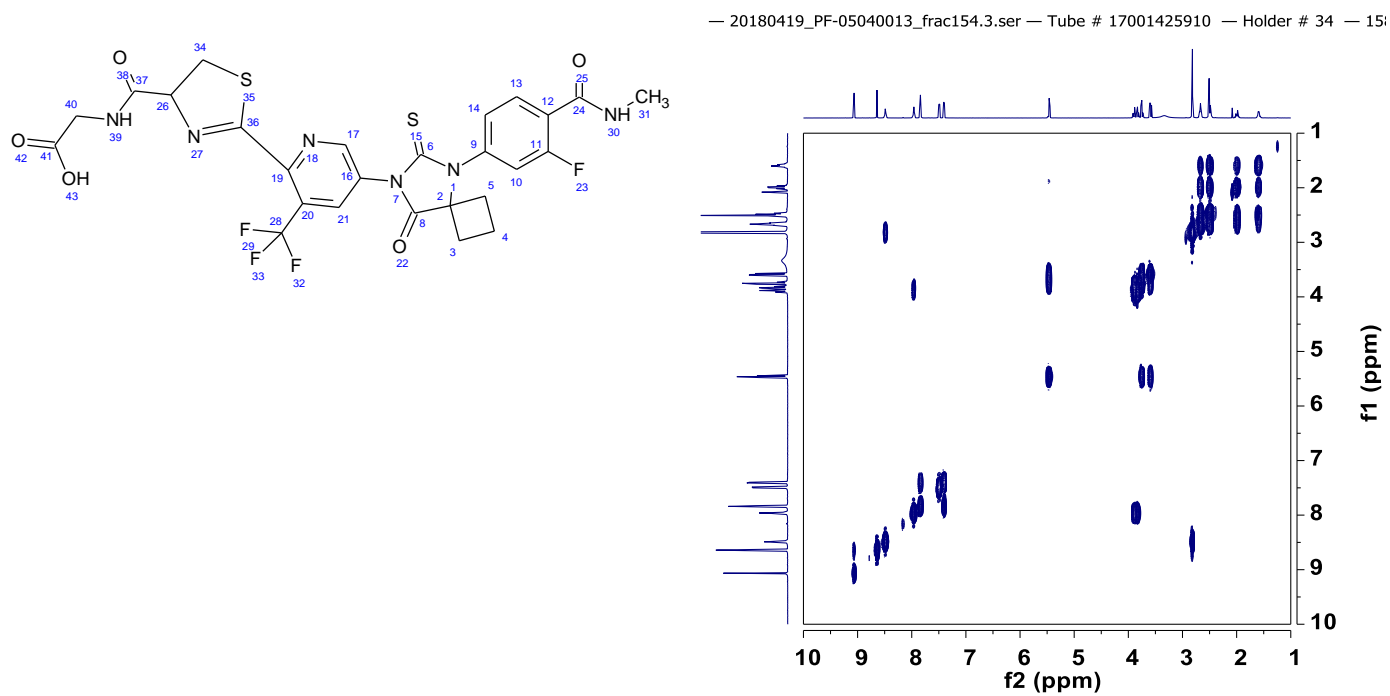
Supplemental Figure 2. The ^1H - ^1H COSY Spectrum of the m/z 583 isolate. COSY, correlated spectroscopy.



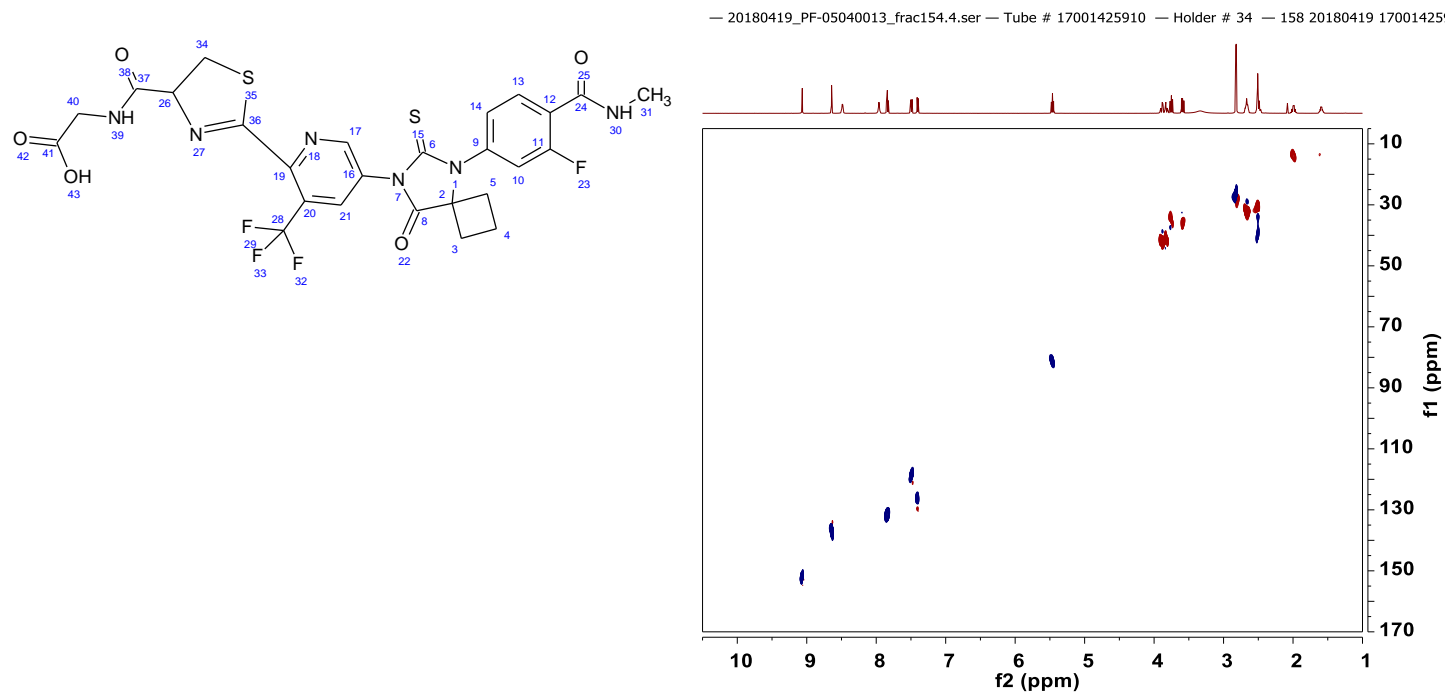
Supplemental Figure 3. The ^1H - ^{13}C multiplicity edited HSQC of the m/z 583 isolate. HSQC, heteronuclear single quantum coherence.



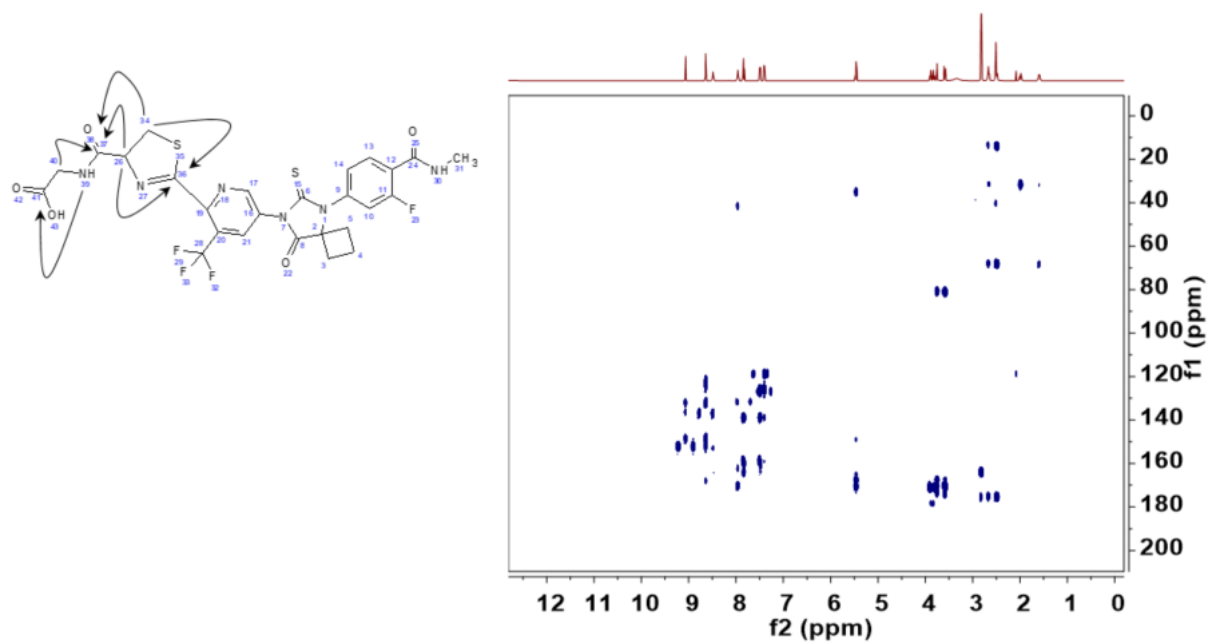
Supplemental Figure 4. The ^1H - ^1H COSY spectrum of the m/z 639 isolate. COSY, correlated spectroscopy.



Supplemental Figure 5. The ^1H - ^{13}C multiplicity edited HSQC of the m/z 639 isolate. HSQC, heteronuclear single quantum coherence.



Supplemental Figure 6. The ^1H - ^{13}C HMBC spectrum of the m/z 639 isolate. HMBC, heteronuclear multiple bond correlation.



Supplemental Information

CEREP assay references from <https://www.eurofinsdiscoveryservices.com/>.

Family	Name	Item
GPCR	5-HT1A Human Serotonin GPCR Binding (Agonist Radioligand) Assay	131
GPCR	5-HT1B Rat Serotonin GPCR Binding (Antagonist Radioligand) Assay	132
GPCR	5-HT2A Human Serotonin GPCR Binding (Agonist Radioligand) Assay	471
GPCR	5-HT2B Human Serotonin GPCR [125I](±)DOI Binding (Agonist Radioligand) Assay	1333
GPCR	5-HT7 Human Serotonin GPCR Binding (Agonist Radioligand) Assay	144
GPCR	A2A Human Adenosine GPCR Binding (Agonist Radioligand) Assay	4
GPCR	alpha1 (Non-Selective) Rat Adrenoceptor GPCR Binding (Antagonist Radioligand) Assay	8
GPCR	alpha2B Human Adrenoceptor GPCR Binding (Antagonist Radioligand) Assay	1344
GPCR	beta1 Human Adrenoceptor GPCR Binding (Agonist Radioligand) Assay	18
GPCR	beta2 Human Adrenoceptor GPCR Binding (Antagonist Radioligand) Assay	20
GPCR	CB1 Human Cannabinoid GPCR Binding (Agonist Radioligand) Assay	36
GPCR	CB2 Human Cannabinoid GPCR Binding (Agonist Radioligand) Assay	37
GPCR	CCK1 (CCKA) Human Cholecystokinin GPCR Binding (Agonist Radioligand) Assay	39
GPCR	CCK2 (CCKB) Human Cholecystokinin GPCR Binding (Agonist Radioligand) Assay	41
GPCR	D1 Human Dopamine GPCR Binding (Antagonist Radioligand) Assay	44
GPCR	D2S Human Dopamine GPCR Binding (Agonist Radioligand) Assay	1322
GPCR	delta (DOP) Human Opioid GPCR Binding (Agonist Radioligand) Assay	114
GPCR	H1 Human Histamine GPCR Binding (Antagonist Radioligand) Assay	870
GPCR	H3 Human Histamine GPCR Binding (Agonist Radioligand) Assay	1332
GPCR	kappa (KOP) Rat Opioid GPCR Binding (Agonist Radioligand) Assay	1971
GPCR	M1 Human Acetylcholine (Muscarinic) GPCR Binding (Antagonist Radioligand) Assay	91
GPCR	M3 Human Acetylcholine (Muscarinic) GPCR Binding (Antagonist Radioligand) Assay	95
GPCR	mu (MOP) Human Opioid GPCR Binding (Agonist Radioligand) Assay	118
GPCR	NK2 Human Tachykinin GPCR Binding (Agonist Radioligand) Assay	102
GPCR	UT (GPR14) Human Urotensin GPCR Binding (Agonist Radioligand) Assay	1386
Ion Channel	5-HT3 Human Serotonin Ion Channel Binding (Antagonist Radioligand) Assay	411

Ion Channel	Cav1.2 (L-type) Rat Calcium Ion Channel Binding (Dihydropyridine Site) Assay	161
Ion Channel	Cav1.2 (L-type) Rat Calcium Ion Channel Binding (Diltiazem Site) Assay	162
Ion Channel	Cav1.2 (L-type) Rat Calcium Ion Channel Binding (Verapamil Site) Assay	163
Ion Channel	Glutamate (AMPA, Non-Selective) Rat Ion Channel [3H] AMPA Binding Assay	64
Ion Channel	Glutamate (Non-Selective) Rat Ion Channel [3H] TCP Binding Assay	124
Ion Channel	nAChR (Muscle-Type) Human Ion Channel Binding (Antagonist Radioligand) Assay	936
Ion Channel	Non-Selective Rat GABAA Ion Channel [35S] TBPS Binding (Antagonist Radioligand) Assay	170
Ion Channel	Non-Selective Rat GABAA Ion Channel [3H] Flunitrazepam Binding (Agonist Radioligand) Assay	28
Ion Channel	Non-Selective Rat Sodium Ion Channel [3H] Batrachotoxinin Binding (Site 2) Assay	169
Neurotransmitter Metabolism	Acetylcholinesterase Human Enzymatic Assay	363
Neurotransmitter Metabolism	Monoamine Oxidase A (MAO-A) Rat Binding (Antagonist Radioligand) Assay	443
Nuclear Hormone Receptor	AR Human Androgen NHR Binding (Agonist Radioligand) Assay	933
Nuclear Hormone Receptor	GR Human Glucocorticoid NHR Binding (Agonist Radioligand) Assay	469
Nuclear Hormone Receptor	PPARgamma Human NHR Binding (Agonist Radioligand) Assay	641
Protease	ACE Human Angiotensin-Converting Metallo Peptidase Enzymatic Assay	3441
Transporter	CHT1 Human Choline Transporter Binding (Antagonist Radioligand) Assay	1552
Transporter	DAT Human Dopamine Transporter Binding (Antagonist Radioligand) Assay	52
Transporter	GABA (Non-Selective) Rat Transporter Binding (Antagonist Radioligand) Assay	60
Transporter	NET Human Norepinephrine Transporter Binding (Antagonist Radioligand) Assay	355
Transporter	SET Human Serotonin Transporter Binding (Antagonist Radioligand) Assay	439

Thermo Fisher Z'-LYTE assay references from <https://www.thermofisher.com/us/en/home/products-and-services/services/custom-services/screening-and-profiling-services/selectscreen-profiling-service/selectscreen-kinase-profiling-service.html>.

ABL1

The 2X ABL1 / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 μ L Kinase Reaction consists of 0.31 - 2 ng ABL1 and 2 μ M

Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

AKT1 (PKB alpha)

The 2X AKT1 (PKB alpha) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.82 - 12 ng AKT1 (PKB alpha) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

AURKA (Aurora A)

The 2X AURKA (Aurora A) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 3.64 ng AURKA (Aurora A) and 2 µM Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

BTK

The 2X BTK / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.04 - 10.4 ng BTK and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

CAMK2A (CaMKII alpha)

The 2X CAMK2A (CaMKII alpha) / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 4 mM CaCl₂, 800 U/ml Calmodulin, 0.02% NaN₃. The final 10 µL Kinase Reaction consists of 0.27 - 1.2 ng CAMK2A (CaMKII alpha) and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 2 mM CaCl₂, 400 U/ml Calmodulin, 0.01% NaN₃. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

CDK2/cyclin A

The 2X CDK2/cyclin A / Ser/Thr 12 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.22 - 10.3 ng CDK2/cyclin A and 2 µM Ser/Thr 12 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

CHEK1 (CHK1)

The 2X CHEK1 (CHK1) / Ser/Thr 19 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.38 - 100 ng CHEK1 (CHK1) and 2 µM Ser/Thr 19 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM

MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:256 dilution of Development Reagent A is added.

CHEK2 (CHK2)

The 2X CHEK2 (CHK2) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 5.51 - 40 ng CHEK2 (CHK2) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

CSNK1A1 (CK1 alpha 1)

The 2X CSNK1A1 (CK1 alpha 1) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 2.6 - 13.5 ng CSNK1A1 (CK1 alpha 1) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

CSNK2A2 (CK2 alpha 2)

The 2X CSNK2A2 (CK2 alpha 2) / Ser/Thr 11 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.6 - 31.5 ng CSNK2A2 (CK2 alpha 2) and 2 µM Ser/Thr 11 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:16 dilution of Development Reagent B is added.

EGFR (ErbB1)

The 2X EGFR (ErbB1) / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 4 mM MnCl₂, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.1 - 8 ng EGFR (ErbB1) and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 2 mM MnCl₂, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

EPHA2

The 2X EPHA2 / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.1 - 40 ng EPHA2 and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

FGFR1

The 2X FGFR1 / Tyr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 4 mM MnCl₂, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 0.45 - 2.45 ng FGFR1 and 2 µM Tyr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 2 mM MnCl₂, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:64 dilution of Development Reagent B is added.

GSK3B (GSK3 beta)

The 2X GSK3B (GSK3 beta) / Ser/Thr 09 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.12 - 0.7 ng GSK3B (GSK3 beta) and 2 µM Ser/Thr 09 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:512 dilution of Development Reagent A is added.

INSR

The 2X INSR / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 4 mM MnCl₂, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1.94 - 50 ng INSR and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 2 mM MnCl₂, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

IRAK4

The 2X IRAK4 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MnCl₂, 1 mM EGTA, 2 mM DTT, 0.02% NaN₃. The final 10 µL Kinase Reaction consists of 3.45 - 63.6 ng IRAK4 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 5 mM MgCl₂, 5 mM MnCl₂, 1 mM EGTA, 1 mM DTT, 0.01% NaN₃. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

JAK3

The 2X JAK3 / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.5 - 3.58 ng JAK3 and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

KDR (VEGFR2)

The 2X KDR (VEGFR2) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.75 - 15 ng KDR (VEGFR2) and 2 µM Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent B is added.

LCK

The 2X LCK / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 8.25 - 100 ng LCK and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

MAP4K4 (HGK)

The 2X MAP4K4 (HGK) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.36 - 3 ng MAP4K4 (HGK) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM

MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

MAPK1 (ERK2)

The 2X MAPK1 (ERK2) / Ser/Thr 03 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2 - 45.5 ng MAPK1 (ERK2) and 2 µM Ser/Thr 03 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPK14 (p38 alpha)

The 2X MAPK14 (p38 alpha) / inactive MAPKAPK2 / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.003 - 0.01 ng MAPK14 (p38 alpha), 6.5 ng inactive MAPKAPK2, and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MAPKAPK2

The 2X MAPKAPK2 / Ser/Thr 04 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.04 - 0.18 ng MAPKAPK2 and 2 µM Ser/Thr 04 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.

MARK1 (MARK)

The 2X MARK1 (MARK) / Ser/Thr 21 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 14.9 - 168 ng MARK1 (MARK) and 2 µM Ser/Thr 21 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

MET (cMet)

The 2X MET (cMet) / Tyr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.49 - 7.84 ng MET (cMet) and 2 µM Tyr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

MST4

The 2X MST4 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 6 - 50 ng MST4 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After

the 1 hour Kinase Reaction incubation, 5 μ L of a 1:45000 dilution of Development Reagent A is added.

MYLK2 (skMLCK)

The 2X MYLK2 (skMLCK) / Ser/Thr 13 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 4 mM CaCl₂, 800 U/ml Calmodulin, 0.02% NaN₃. The final 10 μ L Kinase Reaction consists of 3.13 - 15 ng MYLK2 (skMLCK) and 2 μ M Ser/Thr 13 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 2 mM CaCl₂, 400 U/ml Calmodulin, 0.01% NaN₃. After the 1 hour Kinase Reaction incubation, 5 μ L of a 1:1024 dilution of Development Reagent A is added.

NTRK1 (TRKA)

The 2X NTRK1 (TRKA) / Tyr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 μ L Kinase Reaction consists of 6 - 88.7 ng NTRK1 (TRKA) and 2 μ M Tyr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 μ L of a 1:128 dilution of Development Reagent B is added.

PAK4

The 2X PAK4 / Ser/Thr 20 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 μ L Kinase Reaction consists of 0.1 - 0.75 ng PAK4 and 2 μ M Ser/Thr 20 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 μ L of a 1:256 dilution of Development Reagent A is added.

PDK1

The 2X PDK1 / inactive AKT2 (PKB beta) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 μ L Kinase Reaction consists of 5 - 20 ng PDK1, 150 ng inactive AKT2 (PKB beta), and 2 μ M Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 μ L of a 1:4096 dilution of Development Reagent A is added.

PIM2

The 2X PIM2 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 μ L Kinase Reaction consists of 1.44 - 6.86 ng PIM2 and 2 μ M Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 μ L of a 1:45000 dilution of Development Reagent A is added.

PRKACA (PKA)

The 2X PRKACA (PKA) / Ser/Thr 01 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 μ L Kinase Reaction consists of 0.01 - 0.14 ng PRKACA (PKA) and 2 μ M Ser/Thr 01 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM

MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

PRKCB2 (PKC beta II)

The 2X PRKCB2 (PKC beta II) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 4 mM CaCl₂, 2X Novel Lipid Mix, 0.02% NaN₃. The final 10 µL Kinase Reaction consists of 0.04 - 0.56 ng PRKCB2 (PKC beta II) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 2 mM CaCl₂, 1X Novel Lipid Mix, 0.01% NaN₃. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

ROCK1

The 2X ROCK1 / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.1 - 8 ng ROCK1 and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

SGK (SGK1)

The 2X SGK (SGK1) / Ser/Thr 06 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 0.09 - 1 ng SGK (SGK1) and 2 µM Ser/Thr 06 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:4096 dilution of Development Reagent A is added.

SRC

The 2X SRC / Tyr 02 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 1.54 - 36 ng SRC and 2 µM Tyr 02 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:128 dilution of Development Reagent A is added.

STK3 (MST2)

The 2X STK3 (MST2) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 6.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 0.02% NaN₃. The final 10 µL Kinase Reaction consists of 6.25 - 38.7 ng STK3 (MST2) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.0, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA, 0.01% NaN₃. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

TAOK2 (TAO1)

The 2X TAOK2 (TAO1) / Ser/Thr 07 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 1 mM EGTA. The final 10 µL Kinase Reaction consists of 2.4 - 11 ng TAOK2 (TAO1) and 2 µM Ser/Thr 07 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM

MgCl₂, 1 mM EGTA. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:45000 dilution of Development Reagent A is added.

TEK (Tie2)

The 2X TEK (Tie2) / Tyr 05 mixture is prepared in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 4 mM MnCl₂, 1 mM EGTA, 2 mM DTT. The final 10 µL Kinase Reaction consists of 1 - 6 ng TEK (Tie2) and 2 µM Tyr 05 in 50 mM HEPES pH 7.5, 0.01% BRIJ-35, 10 mM MgCl₂, 2 mM MnCl₂, 1 mM EGTA, 1 mM DTT. After the 1 hour Kinase Reaction incubation, 5 µL of a 1:1024 dilution of Development Reagent A is added.