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

Changhua Ji,† Mausumee Guha,† Xu Zhu,† Jessica Whritenour,‡ Michelle Hemkens,† Susanna Tse,§ Gregory S. Walker,§ Ellen Evans,‡ Nasir K. Khan,‖ Martin B. Finkelstein,‖ Ernesto Callegari,§ and R. Scott Obach§
†Drug Safety Research and Development, Pfizer Inc., La Jolla, CA, USA; ‡Drug Safety Research and Development, Pfizer Inc., Groton, CT, USA; §Pharmacokinetics, Dynamics, and Drug Metabolism, Pfizer Inc., Groton, CT, USA; ‖Drug Safety Research and Development, Pfizer Inc., Pearl River, NY, USA

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-13C 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-13C multiplicity edited HSQC of the m/z 639 isolate. HSQC, heteronuclear single quantum coherence.
Supplemental Figure 6. The 1H-13C HMBC spectrum of the m/z 639 isolate. HMBC, heteronuclear multiple bond correlation.
### Supplemental Information

CEREP assay references from [https://www.eurofinsdiscoveryservices.com/](https://www.eurofinsdiscoveryservices.com/).

<table>
<thead>
<tr>
<th>Family</th>
<th>Name</th>
<th>Item</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPCR</td>
<td>5-HT1A Human Serotonin GPCR Binding (Agonist Radioligand) Assay</td>
<td>131</td>
</tr>
<tr>
<td>GPCR</td>
<td>5-HT1B Rat Serotonin GPCR Binding (Antagonist Radioligand) Assay</td>
<td>132</td>
</tr>
<tr>
<td>GPCR</td>
<td>5-HT2A Human Serotonin GPCR Binding (Agonist Radioligand) Assay</td>
<td>471</td>
</tr>
<tr>
<td>GPCR</td>
<td>5-HT2B Human Serotonin GPCR <a href="%C2%B1">125I</a>DOI Binding (Agonist Radioligand) Assay</td>
<td>1333</td>
</tr>
<tr>
<td>GPCR</td>
<td>5-HT7 Human Serotonin GPCR Binding (Agonist Radioligand) Assay</td>
<td>144</td>
</tr>
<tr>
<td>GPCR</td>
<td>A2A Human Adenosine GPCR Binding (Agonist Radioligand) Assay</td>
<td>4</td>
</tr>
<tr>
<td>GPCR</td>
<td>alpha1 (Non-Selective) Rat Adrenoceptor GPCR Binding (Antagonist Radioligand) Assay</td>
<td>8</td>
</tr>
<tr>
<td>GPCR</td>
<td>alpha2B Human Adrenoceptor GPCR Binding (Antagonist Radioligand) Assay</td>
<td>1344</td>
</tr>
<tr>
<td>GPCR</td>
<td>beta1 Human Adrenoceptor GPCR Binding (Agonist Radioligand) Assay</td>
<td>18</td>
</tr>
<tr>
<td>GPCR</td>
<td>beta2 Human Adrenoceptor GPCR Binding (Antagonist Radioligand) Assay</td>
<td>20</td>
</tr>
<tr>
<td>GPCR</td>
<td>CB1 Human Cannabinoid GPCR Binding (Agonist Radioligand) Assay</td>
<td>36</td>
</tr>
<tr>
<td>GPCR</td>
<td>CB2 Human Cannabinoid GPCR Binding (Agonist Radioligand) Assay</td>
<td>37</td>
</tr>
<tr>
<td>GPCR</td>
<td>CCK1 (CCKA) Human Cholecystokinin GPCR Binding (Agonist Radioligand) Assay</td>
<td>39</td>
</tr>
<tr>
<td>GPCR</td>
<td>CCK2 (CCKB) Human Cholecystokinin GPCR Binding (Agonist Radioligand) Assay</td>
<td>41</td>
</tr>
<tr>
<td>GPCR</td>
<td>D1 Human Dopamine GPCR Binding (Antagonist Radioligand) Assay</td>
<td>44</td>
</tr>
<tr>
<td>GPCR</td>
<td>D2S Human Dopamine GPCR Binding (Agonist Radioligand) Assay</td>
<td>1322</td>
</tr>
<tr>
<td>GPCR</td>
<td>delta (DOP) Human Opioid GPCR Binding (Agonist Radioligand) Assay</td>
<td>114</td>
</tr>
<tr>
<td>GPCR</td>
<td>H1 Human Histamine GPCR Binding (Antagonist Radioligand) Assay</td>
<td>870</td>
</tr>
<tr>
<td>GPCR</td>
<td>H3 Human Histamine GPCR Binding (Agonist Radioligand) Assay</td>
<td>1332</td>
</tr>
<tr>
<td>GPCR</td>
<td>kappa (KOP) Rat Opioid GPCR Binding (Agonist Radioligand) Assay</td>
<td>1971</td>
</tr>
<tr>
<td>GPCR</td>
<td>M1 Human Acetylcholine (Muscarinic) GPCR Binding (Antagonist Radioligand) Assay</td>
<td>91</td>
</tr>
<tr>
<td>GPCR</td>
<td>M3 Human Acetylcholine (Muscarinic) GPCR Binding (Antagonist Radioligand) Assay</td>
<td>95</td>
</tr>
<tr>
<td>GPCR</td>
<td>mu (MOP) Human Opioid GPCR Binding (Agonist Radioligand) Assay</td>
<td>118</td>
</tr>
<tr>
<td>GPCR</td>
<td>NK2 Human Tachykinin GPCR Binding (Agonist Radioligand) Assay</td>
<td>102</td>
</tr>
<tr>
<td>GPCR</td>
<td>UT (GPR14) Human Urotensin GPCR Binding (Agonist Radioligand) Assay</td>
<td>1386</td>
</tr>
<tr>
<td>Ion Channel</td>
<td>5-HT3 Human Serotonin Ion Channel Binding (Antagonist Radioligand) Assay</td>
<td>411</td>
</tr>
</tbody>
</table>
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 μL Kinase Reaction consists of 0.31 - 2 ng ABL1 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.

**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 μL 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 μL Kinase Reaction consists of 3.13 - 15 ng MYLK2 (skMLCK) and 2 μM 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 μL 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 μL Kinase Reaction consists of 6 - 88.7 ng NTRK1 (TRKA) 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.

**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 μL Kinase Reaction consists of 0.1 - 0.75 ng PAK4 and 2 μM 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 μL 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 μL Kinase Reaction consists of 5 - 20 ng PDK1, 150 ng inactive AKT2 (PKB beta), 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.

**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 μL Kinase Reaction consists of 1.44 - 6.86 ng PIM2 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.

**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 μL Kinase Reaction consists of 0.01 - 0.14 ng PRKACA (PKA) 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.

**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.