

WT

~~WT~~

F1031

F1030

	1	2	3	4	5	6	7	8	9	10	11	12
A	0	win										
B	6	win										
C	12	win										
D	18	win										
E	24	win										
F	30	win										
G	36	win										
H	40	win										

F103V

F103W

	1	2	3	4	5	6	7	8	9	10	11	12
A	-	-	-	-	-	-	-	-				
B	0	6	12	18	24	30	36	40				
C	0	6	1	1	1	1	1	1				
D	0	6	1	1	1	1	1	1				
E												
F												
G												
H												

Recipe:

8µl Kaifessant (100µM in 20% DMSO)

2µl Quiklition (0.1M)

8µl 10x Buffer (1M Tris, 100mM NaCl, pH 7.5)

10µl AFOMT (100µM)

1µM STM / SAE

52µl H₂O

avg 70%

F1031 2.8 1:1.4

F103P 2.61 1:1.34

F103V 2.9 1:1.45

F103W 2.37 1:1.35

→ conf 2µl / 10µl → 10µl possible

640 / 1 AFOMT

750µl STM (3.1µM)
 5µl (0.36%)
 • 280µl KA
 • 40µl pen
 • 180µl Buffer
 (310µl AFOMT)
 • 1084µl H₂O

1400x

1, 120 µl Kaifessant

180µl Quiklition

1, 120 µl 10x Buffer

100x

800µl CA

200µl pen

800µl Buffer

70 µM / µl

WT (mucl)

F1031 1.5 357µl + 143

F103P 373µl 127

F103V 344µl 156

F103W 369µl 151

35 x 80 = 2.8µl

3.8 µM STM

280 μ l	K ₂ Cr ₂ O ₇	80
70 μ l	Penetration	20
280 μ l	70x Buffer	80
75 μ l	Stm (sum @ 76%)	20 40 210
1084 μ l	H ₂ O	380 μ l H ₂ O

6.5 μ l / well
with 10 μ l Cr₂O₇ stock

1.4 μ l FeCl₃
- 2.0 μ l FeCl₃/H₂O
1.4 μ l Stm (sum @ 76%)
1.75 μ l H₂O

80 μ l

1 μ l
150 μ l FeCl₃
10 μ l Stm
840 μ l H₂O

50 μ l Assay

+ 20 μ l TCA - 10% TCA;
(OMT-stop) 50% MeCN)

rt, eff., 10 min GS

→ 10 min, eff 4°C, 14000x

↓ Überstände → GPC

C18: A) MeCN / H₂PO₄ (0,5%)

~~10%~~ B) MeCN + 0,2% HCOOH / H₂O + 0,2% FA

2% → 100%

10% TCA - 10% C₁₈ in H₂O)

2 x 100 μ l EtOAc extrahieren

Überstände sammeln

CM Sepher, in 100 μ l MeCN
100% MeCN