

PFOMT-Kristallisation 28.10.2013

1 μ l + 1 μ l

Reservoir: 100 mM Hepes/NaOH, 0.2 M CaCl_2
→ with pH 6.5-7.5 and 15-25% (w/V) PEG-4000

Other solutions:

- 250 mM MgCl_2 in ddH₂O
- 250 mM Quercetin in DMSO
- 250 mM Eriodictyol in DMSO

wt - 16.12 $\frac{\text{mg}}{\text{ml}}$
Var. ~~12.74~~ 14.81
Y51R N202W → E=0.1%
91 222
0.858 $\frac{\text{mg}}{\text{ml}}$ (Var)
0.71 (wt)

1. Concentrate Protein in centrifugal filter and rebuffer into 10 mM Tris/HCl pH 7.5
2. Readjust concentration to 6.3 mg/mL with 10 mM Tris/HCl pH 7.5

For crystallization of:

- A) Original (Paper, only wt):** add 1:1000 250mM MgCl_2 (final 250 μ M), 1:10.000 250 mM Quercetin (final 25 μ M), 1:20 5 mM SAM (final 250 μ M)
- B) Without substrate (only wt):** add 1:1000 250mM MgCl_2
- C) SAE + ED (wt and variant):** add 1:1000 250mM MgCl_2 (final 250 μ M), 1:1000 250 mM Eriodictyol (final 250 μ M), 1:20 5 mM SAE (final 250 μ M)
- D) SAE + Q (wt and variant):** add 1:1000 250mM MgCl_2 (final 250 μ M), 1:1000 250 mM Quercetin (final 250 μ M), 1:20 5 mM SAE (final 250 μ M)

E) SAE - 250 μ M MgCl_2 , 250 μ M SAM

	1	2	3	4	5
A	pH 6.5 15% PEG	pH 6.5 20% PEG	<i>Prec</i> 2>1 pH 6.5 25% PEG	pH 6.5 15% PEG	<i>Prec</i> 2>1 pH 6.5 20% PEG
B	pH 7.0 15% PEG	pH 7.0 20% PEG	<i>Prec.</i> 2>1 pH 7.0 25% PEG	pH 7.0 15% PEG	<i>Prec</i> 2>1 pH 7.0 20% PEG
C	pH 7.5 15% PEG	pH 7.5 20% PEG	<i>Precip</i> 2>1 pH 7.5 25% PEG	pH 7.5 15% PEG	<i>Prec</i> 2>1 pH 7.5 20% PEG

	1	2	3	4	5
A	<i>+Prec</i> pH 6.5 25% PEG	pH 6.5 15% PEG	pH 6.5 20% PEG	pH 6.5 25% PEG	<i>Krist?</i> <i>Prec</i> pH 6.5 15% PEG
B	<i>+Prec</i> pH 7.0 25% PEG	<i>keine Krist?</i> pH 7.0 15% PEG	pH 7.0 20% PEG	<i>Prec</i> pH 7.0 25% PEG	<i>-Prec</i> pH 7.0 15% PEG
C	<i>+Prec</i> pH 7.5 25% PEG	pH 7.5 15% PEG	<i>1 Krist</i> <i>in PEG 20%</i> pH 7.5 20% PEG	<i>Prec</i> pH 7.5 25% PEG	<i>-Prec</i> pH 7.5 15% PEG

A) wt - 250 μ M MgCl₂,
25 μ M Quercetin,
250 μ M SAM, 2,5% DMSO

B) wt - ~~250 μ M MgCl₂~~
– no other effectors (e.g. substrates)

C1) Wt - 250 μ M MgCl₂, 250 μ M SAE,
250 μ M ED

C2) Variante - 250 μ M MgCl₂,
250 μ M SAE, 250 μ M ED

Prot & Effekt *Resonanz*
↓ ↓

① 1:1:1

② 1:1:2

Prot *Res*
① 1 1

② 1 2

	1	2	3	4	5
A	<i>Proc</i> pH 6.5 20% PEG	<i>Krist</i> <i>→ Proc</i> pH 6.5 25% PEG	pH 6.5 15% PEG <i>Krist</i>	pH 6.5 20% PEG	<i>Proc</i> pH 6.5 25% PEG
B	<i>Proc</i> pH 7.0 20% PEG	pH 7.0 25% PEG	pH 7.0 15% PEG	pH 7.0 20% PEG	<i>Proc</i> pH 7.0 25% PEG
C	<i>Proc?</i> <i>→ Krist</i> pH 7.5 20% PEG	pH 7.5 25% PEG	pH 7.5 15% PEG	<i>Krist</i> pH 7.5 20% PEG	<i>Krist</i> <i>Proc</i> pH 7.5 25% PEG

C2) Var - 250 μ M MgCl₂, 250 μ M SAE,
250 μ M ED

D) wt - 250 μ M MgCl₂, 250 μ M ~~SAE~~,
~~250 μ M Quercetin~~ *SAE*

Wt → 1:2,688 → ant 12 ug/ml VV
 Var → 1: ~~2.96~~ 2.98 → ant 12 ug/ml VV

A) 208 µl total
 10 µl Protein
 + 10 µl (A)
~~+ 6 µl Tris~~

Butter
~~10 µl~~
~~98~~
~~988~~
18 µl (A)
 2 µl Quercetin
 20 µl 250 mM HgCl_2
 100 µl SAM
 948 µl Tris
 1 µl (B)
2 µl HgCl_2
 998 µl Tris buffer

(C) 1 µl
2 µl HgCl_2
2 µl Bischofsgel.
 50 µl SAE
 948 µl Tris

(E)
 1 µl HgCl_2
 50 µl SAE
 948 µl Tris
 1:105 VV
 19:20

1:20

1:19

19:20

• ant 12 ug/ml Quercetin, soles
 Kristallisations lösg:

10 µl Prot (12 ug/ml)
 + 10 µl Effektore
 → 6 ug/ml total

4x Effektorlösung

A) 1 µl Quercetin (25 mM) ✓
 1 µl HgCl_2 (250 mM) ✓
 50 µl SAM (5 mM) ✓
 ad to 250 µl Tris (198) ✓
 final conc
 0,1 mM
 1 mM
 1 mM

A) 1 µl Quercetin (2,5 mM)
 1 µl HgCl_2 (25 mM)
 5 µl SAM 5 mM.

B) 1 µl HgCl_2
 ad to 250 µl Tris (249) 1 mM

C) 1 µl HgCl_2 (250 mM) ✓
 1 µl Bischofsgel (250 mM) ✓
 50 µl SAE (5 mM) ✓
 ad to 250 µl Tris (198) 1 mM

250ml
10mM Tris / HCl
pH 7.5

250ml
10mM Tris / HCl
pH 7

~~250ml~~
~~20% PEG 4000~~

for 50ml
10mM Hepes pH 6.5 / 7 / 7.5
15/20/25% PEG
0.2M CaCl_2

~~50ml~~
~~10mM Hepes pH 7~~

~~10ml~~
20mM Hepes
0.4M CaCl_2

Paper X-tal Buffer

20% PEG 4000
0.2M CaCl_2
100mM Hepes / NaOH
pH 7

100ml
40mM Hepes
0.8M CaCl_2
0.95
4.3g Hep
47.6g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$
11.76

3x
→ 25ml
ant pH 6.5 / 7 / 7.5
+ 25ml ddH₂O

for 50ml
20mM Hepes
0.4mM CaCl_2
pH 6.5 / 7 / 7.5

↓
for 3x 5ml
+ 15/20/25% PEG
+ 25ml ddH₂O

for 50ml
20mM Hepes
0.2mM CaCl_2
15/20/25 PEG
pH 6.5 / 7 / 7.5

25% 5ml 20mM Hepes (pH x)
+ 5ml PEG (50%)
+ 4ml PEG (50%)
20% + 1ml ddH₂O

5ml Hepes
15% + 8ml PEG
2ml ddH₂O