

## SOP\_hymanLab-04 – XMAP215 purification

Authors: Widlund et al. (Doi: <https://10.1073/pnas.1016498108>)

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## I. List of abbreviations

%	Percent
°C	Degree Celsius
µg	Micrograms
µL	Microliter
BIIC	Baculovirus infected insect cell
CV	Column Volume
DTT	Dithiothreitol
H <sub>2</sub> O	Water
KCl	Potassium Chloride
L	Liters
MES	2-(N-morpholino)ethanesulfonic acid
min	Minutes
mL	Milliliter
mM	Millimolar
PMSF	Phenylmethylsulfonylfluorid
rpm	Rounds per minute
RT	Room temperature
X g	Gravity

## 1. Aim

This SOP is a systematic and written instruction on the procedure of XMAP215 protein purification in the laboratory of Prof. Anthony A. Hyman, Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany.

## 2. Background

XMAP215/Dis1 family proteins positively regulate microtubule growth. The current protocol for the purification of XMAP215 has been adapted from Widlund et al (2011) <sup>i</sup>. The coding region of XMAP215 was originally modified by addition of either a C-terminal His7 tag or a C-terminal enhanced green fluorescent protein (GFP) His7 tag and cloned into the pFastBac1 vector. From these constructs, we generated Baculovirus (Bac-to-Bac system, Invitrogen) that was subsequently used to infect SF+ cells. This purification usually gives up to 5 mg protein per Liter of cells infected.

### 3. Procedure

#### 3.1 Buffers

##### 3.1.1 Lysis buffer

Chemical	Concentration (mM)
HEPES pH 7.5	50
NaCl	50
Glycerol	5 %
Triton-X	0.1 %
PMSF	0.1
Protease Inhibitor cocktail (Roche)	1 tablet per 50 mL buffer
CaCl <sub>2</sub>	10

##### 3.1.2 Cation buffer

Chemical	Concentration (mM)
HEPES pH 7.5	6.7
NaCl	200
MES	6.7
Na-acetate	6.7

##### 3.1.3 Cation Wash buffer I

Chemical	Concentration (mM)
HEPES pH 7.5	6.7
NaCl	75
MES	6.7
Na-acetate	6.7

##### 3.1.4 Cation Wash buffer II

Chemical	Concentration (mM)
HEPES pH 7.5	6.7
NaCl	150
MES	6.7
Na-acetate	6.7

##### 3.1.5 Cation Elution buffer

Chemical	Concentration (mM)
HEPES pH 7.5	6.7
NaCl	600
MES	6.7
Na-acetate	6.7

##### 3.1.6 Imidazole buffer

<b>Chemical</b>	<b>Concentration (mM)</b>
Tris pH 8.0	50
NaCl	300
Glycerol	10 %
Imidazole	15

### 3.1.7 Imidazole wash buffer I

<b>Chemical</b>	<b>Concentration (mM)</b>
Tris pH 8.0	50
NaCl	300
Glycerol	10 %
Imidazole	30

### 3.1.8 Imidazole wash buffer II

<b>Chemical</b>	<b>Concentration (mM)</b>
Tris pH 8.0	50
NaCl	300
Glycerol	10 %
Imidazole	60

### 3.1.9 Imidazole elution buffer

<b>Chemical</b>	<b>Concentration (mM)</b>
Tris pH 8.0	50
NaCl	300
Glycerol	10 %
Imidazole	600

### 3.1.10 SEC elution buffer

<b>Chemical</b>	<b>Concentration (mM)</b>
Tris pH 6.6	10
Bis-Tris	10
KCl	100
Glycerol	10 %
DTT	1

### 3.2 Insect cell transfection and lysis

- 3.2.1 Infect SF+ ( $10^6$  cell/mL) cells in 500 mL medium with 1 % P2 Baculovirus stock
- 3.2.2 After 72 h, harvest cells by centrifuging at 1700 rpm for 15 min in a Hereaus Megafuge centrifuge
- 3.2.3 Resuspend cells using 50 mL of ice-cold **Lysis buffer** for every 500 mL of cells
  - If cells need to be stored, exclude the protease inhibitor cocktail tablet, PMSF and CaCl<sub>2</sub> from the lysis buffer and snap freeze cells in liquid nitrogen
  - To continue the purification, thaw cells and add a complete protease inhibitor cocktail tablet (Roche), 0.1 mM PMSF and 10 mM CaCl<sub>2</sub>
- 3.2.4 Lyse cells using the LM20 microfluidizer with 2 passages at 15,000 psi
- 3.2.5 Clear crude lysate by centrifugation at 80,000 rpm for 45 minutes at 15 °C in a Beckman Ultramax centrifuge with a MLA-80 rotor – Collect 50 µL – Sample 1

### 3.3 SP sepharose column purification (5 ml HiTrap SP HP (GE Healthcare), peristaltic pump at RT)

- 3.3.1 Rinse the column with 10 CV of H<sub>2</sub>O at 5 mL/min
- 3.3.2 Equilibrate the column with 10 CV **Cation buffer** at 5 mL/min
- 3.3.3 Load crude supernatant (sample 1) at 2.5 mL/min – Collect 50 µL of flow through – Sample 2
- 3.3.4 Rinse the column with 10 CV **Cation Wash buffer I** at 5 ml/min – Collect 50 µL of flow through - Sample 3
- 3.3.5 Rinse the column with 10 CV **Cation Wash buffer II** at 5 ml/min – Collect 50 µL of flow through - Sample 4
- 3.3.6 Elute 1.5 ml fractions with **Cation Elution buffer** at 5 ml/min
- 3.3.7 Pool fractions containing the protein in **Imidazole buffer** after confirmation by Bradford – Collect 50 µL – Sample 5

### 3.4 Ni-NTA sepharose His-Trap HP column purification (5 ml His-Trap HP column (GE Healthcare), peristaltic pump at RT)

- 3.4.1 Rinse the column with 10 CV of H<sub>2</sub>O at 5 mL/min
- 3.4.2 Equilibrate the column with 10 CV **Imidazole buffer** at 5 mL/min
- 3.4.3 Load pooled protein fractions (sample 5) at 1 mL/min – Collect 50 µL of flow through – Sample 6

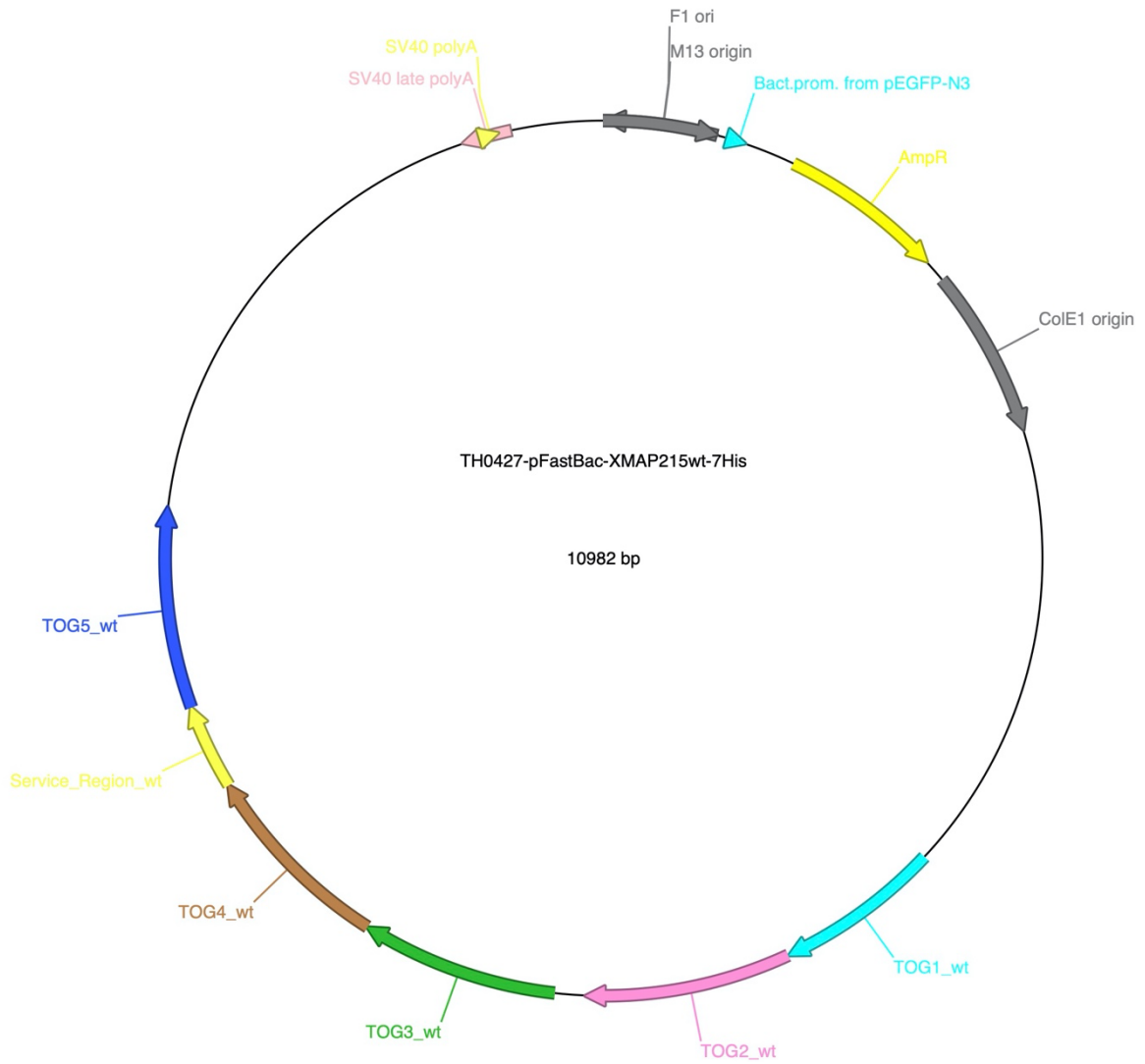
- 3.4.4 Rinse the column with 10 CV **Imidazole wash buffer I** at 2.5 ml/min – Collect 50  $\mu$ L of flow through -Sample 7
- 3.4.5 Rinse the column with 10 CV **Imidazole wash buffer II** at 2.5 ml/min – Collect 50  $\mu$ L of flow through - Sample 8
- 3.4.6 Elute 1.5 ml fractions with **Imidazole elution buffer** at 5 ml/min
- 3.4.7 Pool fractions containing the protein after confirmation by Bradford – Collect 50  $\mu$ L - Sample 9

### 3.5 Size exclusion chromatography (Superdex 200 16/60 (GE Healthcare), AKTA pure at RT)

- 3.5.1 Equilibrate column using 10 CV **SEC elution buffer** at 5 mL/min
- 3.5.2 Load sample 9 on column and collect peak fractions
- 3.5.3 Pool fractions and concentrate to at least 6  $\mu$ M using an Amicon Ultra 10 K MWCO concentrator (Millipore) and add Glycerol to 10 % v/v final

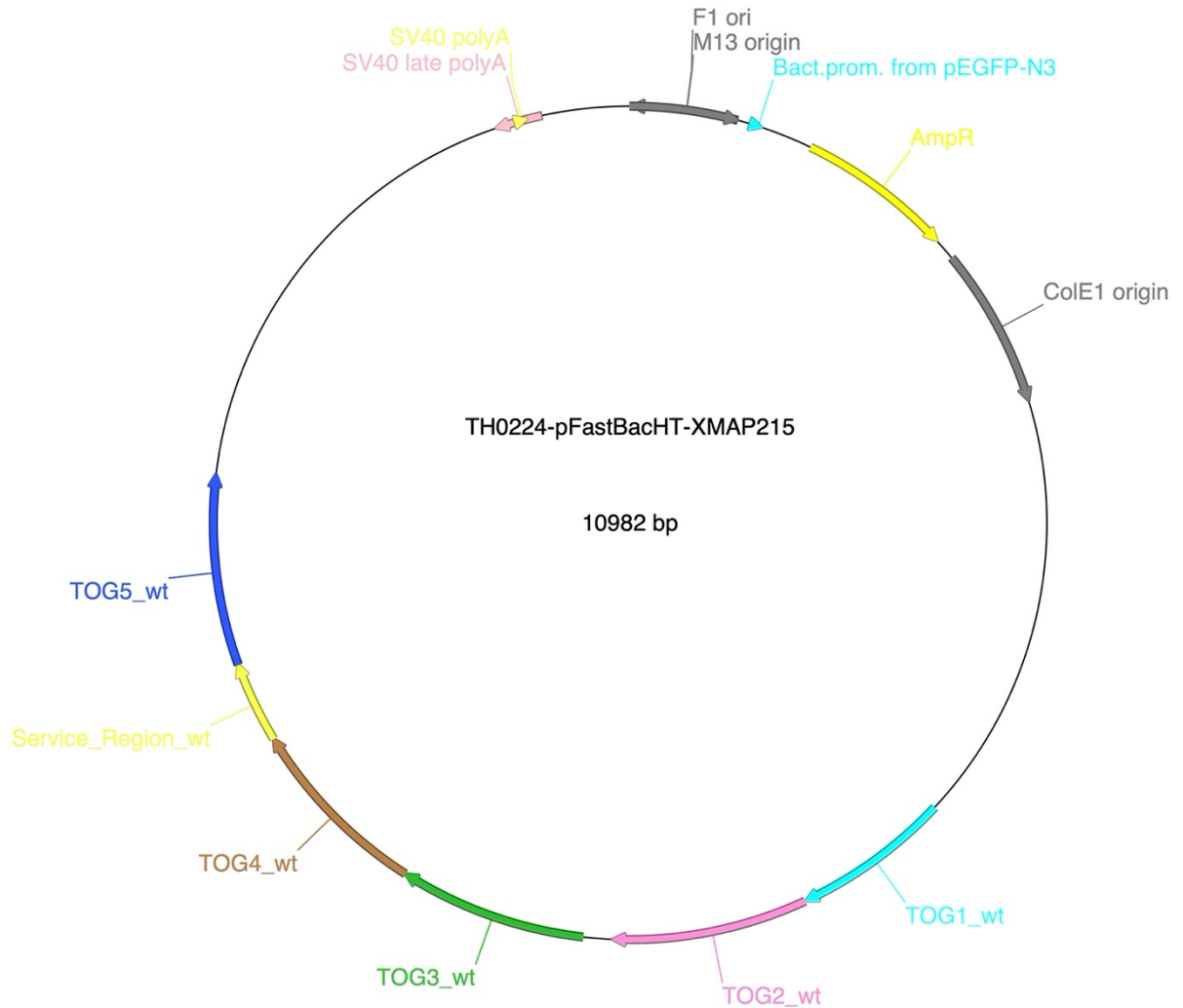
#### 4. Attachments:

##### 4.1 TH0427-pFastBac-XMAP215wt-7His





## 4.2 TH0224-pFastBacHT-XMAP215



## 5. Change history

Version....from	Page(s)	Changes/ additions

## 6. References

<sup>i</sup> Widlund, P. O., Stear, J. H., Pozniakovsky, A., Zanic, M., Reber, S., Brouhard, G. J., et al. (2011). XMAP215 polymerase activity is built by combining multiple tubulin-binding TOG domains and a basic lattice-binding region. *Proceedings of the National Academy of Sciences*, 108(7), 2741–2746. doi:10.1073/pnas.1016498108