



SEP 23, 2023

OPEN ACCESS



DOI:
[dx.doi.org/10.17504/protocol
s.io.q26g7pbo1gwz/v1](https://dx.doi.org/10.17504/protocols.io.q26g7pbo1gwz/v1)

Protocol Citation: Elias
Adriaenssens 2023.
Purification of GST-tagged
linear tetra-ubiquitin (4xUb).
protocols.io
[https://dx.doi.org/10.17504/p
rotocols.io.q26g7pbo1gwz/v1](https://dx.doi.org/10.17504/p
rotocols.io.q26g7pbo1gwz/v1)

License: This is an open
access protocol distributed
under the terms of
the [Creative Commons
Attribution License](#), which
permits unrestricted use,
distribution, and reproduction
in any medium, provided the
original author and source
are credited

Protocol status: Working
We use this protocol and it's
working

Created: Jul 07, 2023

Last Modified: Sep 23,
2023

PROTOCOL integer ID:
84631


Keywords: GST-tagged
linear tetra-ubiquitin,
purification

Purification of GST-tagged linear tetra-ubiquitin (4xUb)

Elias Adriaenssens¹

¹Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

ASAP Collaborative Research Network Sascha Martens lab, University of Vienna, Max Perutz Labs - Vienna

 Elias Adriaenssens
Sascha Martens lab, University of Vienna, Max Perutz Labs - ...

ABSTRACT

This protocol describes purification of GST-tagged linear tetra-ubiquitin (4xUb).

ATTACHMENTS

[774-1960.pdf](#)

MATERIALS

Materials

- pGEX-4T1 vector (RRID:Addgene #199779)
- isopropyl β-D-1-thiogalactopyranoside (IPTG)
- Glutathione Sepharose 4B beads (GE Healthcare)
- 10 kDa cut-off Amicon filter (Merck Millipore)
- Superdex 200 Increase 10/300 GL column (Cytiva)
- SORVAL RC6+ centrifuge with an F21S8x50Y rotor (Thermo Scientific)

Lysis buffer

A	B
Tris-HCl pH 7.4	50 mM
NaCl	300 mM
MgCl2	2 mM
DTT	1 mM
cOmplete EDTA-free protease inhibitors (Roche)	
DNase (Sigma)	

Wash buffer

A	B
Tris-HCl pH 7.4	50 mM
NaCl	300 mM
DTT	1 mM

High salt wash buffer

A	B
Tris-HCl pH 7.4	50 mM

A	B
NaCl	700 mM
DTT	1 mM



SEC buffer

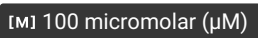


A	B
Tris-HCl pH 7.4	25 mM
NaCl	150 mM
DTT	1 mM

Purification of GST-tagged linear tetra-ubiquitin (4xUb)

18h 46m

1 Linear tetra-ubiquitin fused to GST (GST-4xUb) was cloned into a pGEX-4T1 vector and is available from Addgene (RRID:Addgene #199779).

2 After the transformation of the pGEX-4T1 vector encoding GST4xUb in *E. coli* Rosetta pLySS cells, grow cells in LB medium at  37 °C until an OD₆₀₀ of 0.4 and then continued at  18 °C.

3 Once the cells reached an OD₆₀₀ of 0.8, induce protein expression with  100 micromolar (μM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for  16:00:00 at  18 °C.

16h

4 Collect cells by centrifugation and resuspend in lysis buffer.



5 Sonicate cell lysates.

5.1 Sonicate cell lysates for  00:00:30. (1/2)

30s

5.2 Sonicate cell lysates for 00:00:30 (2/2)

30s

6 Clear lysates by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S8x50Y rotor (Thermo Scientific).

45m

7 Collect the supernatant and incubate with preequilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind GST-4xUb.

2h

8 Centrifuge the samples to pellet the beads and remove the unbound lysate.

9 Wash the beads.

9.1 Wash the beads twice with wash buffer.

9.2 Wash the beads once with high salt wash buffer.

9.3 Wash the beads twice with wash buffer.

10 Incubate the beads Overnight with 4 mL of 50 millimolar (mM) reduced glutathione dissolved in wash buffer at 4 °C, to elute GST-4xUb from the beads.

11 To collect the supernatant, collect the beads by centrifugation.

12 Wash the beads twice with 4 mL of wash buffer, and collect the supernatant.



13 Pool the supernatant fractions, filter through a $0.45\ \mu\text{m}$ syringe filter, concentrated with 10 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

14 Elute the proteins with SEC buffer.

15 Analyze the fractions by SDS-PAGE and Coomassie staining.



16 Pool the fractions containing purified GST-4xUb.

17 After concentrating the purified protein, aliquot the protein and snap-freeze in liquid nitrogen. Store the proteins at $-80\ ^\circ\text{C}$.