Aug 29, 2024

Purification of BCL2L13-GFP

DOI
dx.doi.org/10.17504/protocols.io.36wgqno2ogk5/v1

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Protocol Citation: Elias Adriaenssens 2024. Purification of BCL2L13-GFP. **protocols.io** **https://dx.doi.org/10.17504/protocols.io.36wgqno2ogk5/v1**

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

Created: May 24, 2024

Last Modified: August 29, 2024

Protocol Integer ID: 103090

Keywords: ASAPCRN

Funders Acknowledgement:
Aligning Science Across Parkinson's (ASAP)
Grant ID: ASAP-000350
Marie Skłodowska-Curie MSCA Postdoctoral fellowship
Grant ID: 101062916

Abstract

This protocol details the purification of BCL2L13-GFP.

Materials

Lysis buffer:

	A	B
	Tris-HCl	50 mM
	pH	7.4
	NaCl	300 mM
	Triton X-100	1%
	glycerol	5%
	MgCl2	2 mM
	DTT	1 mM
	β-mercaptoethanol	2mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

Wash buffer:

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

High salt wash buffer:

	Tris-HCl	50 mM
	pH	7.4
	NaCl	700 mM
	DTT	1 mM

SEC buffer:

	Tris-HCl	25 mM
	pH	7.4
	NaCl	300 mM
	DTT	1 mM

- pET-DUET1 vector (available on Addgene) pETDuet-1 TIM9,10 **addgene Catalog #**170280
- BCL2L13 W275A/I278A (ΔLIR1)(available on Addgene)
- BCL2L13 Y213A/I216A/W275A/I278A (ΔLIR1+2) (available on Addgene)
- BCL2L13 I224A/L227A/W275A/I278A (ΔLIR1+3) (available on Addgene)
- BCL2L13 W275A/I278A/I307A/V310A (ΔLIR1+4) (available on Addgene)
- BCL2L13 I224A/L227A/W275A/I278A/I307A/V310A (ΔLIR1+3+4) (available on Addgene)
- Rosetta pLysS cells (Novagen Cat# 70956-4)
  Rosetta™(DE3)pLysS Competent Cells - Novagen **Merck Catalog #**70956-4
- SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific)
- Glutathione Sepharose 4B beads (GE Healthcare)
- 10 kDa cut-off Amicon filter (Merck Millipore)
  Amicon® Ultra Centrifugal Filter, 10 kDa MWCO **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**UFC801008
- GFP-tagged BCL2L13-GFP (available from Addgene),
- BCL2L13(W275A/I278A)-GFP (ΔLIR1) (available from Addgene),
- BCL2L13(Y213A/I216A/W275A/I278A)-GFP (ΔLIR1+2) (available from Addgene),
- BCL2L13(I224A/L227A/W275A/I278A)-GFP (ΔLIR1+3)(available from Addgene),
- BCL2L13(W275A/I278A/I307A/V310A)-GFP (ΔLIR1+4) (available from Addgene),
- BCL2L13(I224A/L227A/W275A/I278A/I307A/V310A)-GFP (ΔLIR1+3+4)(available from Addgene)

Purification - BCL2L13-GFP

20h 46m

1 To purify GFP-tagged

- BCL2L13-GFP (available from Addgene),
- BCL2L13(W276A/I279A)-GFP (ΔLIR1) (available from Addgene),
- BCL2L13(Y213A/I216A/W276A/I279A)-GFP (ΔLIR1+2) (available from Addgene),
- BCL2L13(I224A/L227A/W276A/I279A)-GFP (ΔLIR1+3)(available from Addgene),
- BCL2L13(W276A/I279A/I307A/V310A)-GFP (ΔLIR1+4) (available from Addgene),
- BCL2L13(I224A/L227A/W276A/I279A/I307A/V310A)-GFP (ΔLIR1+3+4)(available from Addgene),

fuse the cytosol-exposed domain of BCL2L13 (1-463aa) to a C-terminal GFP-tag through cloning into a pET-DUET1 vector (available on Addgene).

2 Introduce the point mutants by in vitro mutagenesis to generate

- BCL2L13 W276A/I279A (ΔLIR1)(available on Addgene),
- BCL2L13 Y213A/I216A/W276A/I279A (ΔLIR1+2) (available on Addgene),
- BCL2L13 I224A/L227A/W276A/I279A (ΔLIR1+3) (available on Addgene),
- BCL2L13 W276A/I279A/I307A/V310A (ΔLIR1+4) (available on Addgene),
- BCL2L13 I224A/L227A/W276A/I279A/I307A/V310A (ΔLIR1+3+4) (available on Addgene).

3 After the transformation of the pET-DUET1 vector encoding BCL2L13-GFP wild-type or mutants in E. coli Rosetta pLysS cells (Novagen Cat# 70956-4), grow the cells in 2x Tryptone Yeast extract (TY) medium at 37 °C until an OD_{600} of 0.4 and then continued at 18 °C.

4 Once the cells reaches an OD_{600} of 0.8, induce the protein expression with 100 micromolar (μM) isopropyl β-D-1-thiogalactopyranoside (IPTG) for 16:00:00 at 18 °C. Collect the cells by centrifugation and resuspend in lysis buffer.

16h

Lysis buffer:

	A	B
	Tris-HCl pH 7.4	50 mM
	NaCl	300 mM
	Triton X-100	1%
	Glycerol	5%
	MgCl2	2 mM
	DTT	1 mM

	A	B
	β-mercaptoethanol	2mM
	cOmplete EDTA-free protease inhibitors (Roche)	
	CIP protease inhibitor (Sigma)	
	DNase (Sigma)	

5 Sonicate the cell lysates twice for 30 s and clear by centrifugation at 18000 rpm, 4°C, 00:45:00 in a SORVAL RC6+ centrifuge with an F21S-8x50Y rotor (Thermo Scientific). 45m

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

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

6 Collect the supernatant and incubate with pre-equilibrated Glutathione Sepharose 4B beads (GE Healthcare) for 02:00:00 at 4 °C with gentle shaking to bind BCL2L13-GFP. 2h

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

8 Wash the beads twice with wash buffer, once with high salt wash buffer, and two more times with wash buffer.

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
	NaCl	700 mM
	DTT	1 mM

9 To cleave off the GST-tag Overnight, elute the GFP-tagged cargo receptor from the GSH beads by the addition of TEV protease.

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

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

12 Pool the supernatant fractions, filter through a 0.45 µm syringe filter, concentrate with 50 kDa cut-off Amicon filter (Merck Millipore), and load onto a pre-equilibrated Superdex 200 Increase 10/300 GL column (Cytiva).

13 Elute the proteins with SEC buffer.

SEC buffer:

	A	B
	Tris-HCl pH 7.4	25 mM
	NaCl	300 mM
	DTT	1 mM

14 Analyze fractions by SDS-PAGE and Coomassie staining. Pool fractions containing purified BCL2L13-GFP.

15 After concentrating the purified protein, aliquote the protein and snap-frozen in liquid nitrogen.

Note

Store the proteins at -80 °C.