



Aug 22, 2021

WIPI2d Expression and purification

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ABSTRACT

WIPI2d expression and purification from HEK cells

DOI

dx.doi.org/10.17504/protocols.io.buxqnxmw

PROTOCOL CITATION

Imstrong 2021. WIPI2d Expression and purification. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.buxqnxmw>



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CREATED

May 11, 2021

LAST MODIFIED

Aug 22, 2021

PROTOCOL INTEGER ID

49872

Expression 2d 12h 50m

- 1 Transfect 300 mL of HEK GNTI cells
- 2 Dilute PEI with Warm Hybridoma-SFM(1X)
- 3 In a separate tube, dilute DNA with Hybridoma-SFM(1X)
- 4 Add PEI to DNA dilution. Incubate mixture for 00:30:00 at 37 °C

30m

- 5 Add mixture to cells. Let cells grow for ⌚ **60:00:00** 2d 12h
- 6 Harvest Cells 🌀 **2500 rpm, 4°C, 00:20:00** 20m
- 7 Wash pellet with cold PBS. Store pellet until Purification.

Purification 2d 12h 50m

- 8 Resuspended pellet in 25mL of lysis buffer (50mM Hepes pH 7.45, 300mM NaCl, 1mM TCEP, 1% Triton X, PI cocktail)
- 9 Let rock at 🌡 **4 °C** for ⌚ **00:20:00** 20m
- 10 Clarify lysate for 🌀 **18000 rpm, 4°C, 00:30:00** 30m
- 11 Rock supernatant with equilibrated strep resin for ⌚ **02:00:00** at 🌡 **4 °C** 2h
- 12 Let supernatant FT gravity column
- 13 Wash with 50CV wash buffer (50mM Hepes pH 7.45, 300mM NaCl, 1mM TCEP)
- 14 Elute 2X with 5mL of wash buffer spiked with 5mM des-thiobiotin
- 15 Concentrate elution and inject onto pre-equilibrated S200 10/30 column (25mM Hepes pH 7.45, 150mM NaCl, 1mM TCEP)
- 16 Pool peak fractions, concentrate, snap freeze, and store at -80C