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pA-Hia5 Protein Expression and Purification

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ABSTRACT

This is a protocol for the purification of protein A-Hia5 (pA-Hia5), protein AG-Hia5 (pAG-Hia5), and/or the Hia5 MTase. It was adapted from the protein purification protocol used by Stergachis et al 2020, but significant changes have been made following the His column purification. These proteins can be used for Directed Methylation and Long-read sequencing (DiMeLo-seq), as outlined in the "DiMeLo-seq: Directed Methylation with Long-read sequencing" on protocols.io.

The protocol will refer to pA-Hia5 throughout for the sake of simplicity, but the same instructions apply for pA-Hia5, pAG-Hia5, and Hia5.

Stergachis AB, Debo BM, Haugen E, Churchman LS, Stamatoyannopoulos JA (2020). Single-molecule regulatory architectures captured by chromatin fiber sequencing.. Science (New York, N.Y.).
https://doi.org/10.1126/science.aaz1646

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MATERIALS TEXT

T7 Express lysY competent E. coli cells (NEB #C3010I)

Kanamycin [M]**50 mg/ml** (Sigma-Aldrich BP861)

Chloramphenicol [M]**34 mg/ml** (Fisher IB02080)

IPTG [M]**1 Molarity (M)** (prepare at least **6 mL**) (Fisher 15529019)

Lysis buffer (prepare at least **250 mL**)

- HEPES [M]**50 Milimolar (mM)** pH**7.5** (Sigma-Aldrich 7365-45-9)
- NaCl [M]**300 Milimolar (mM)** (Sigma-Aldrich 7647-14-5)
- Glycerol [M]**10 % (v/v)** (Fisher 17904)
- Triton X-100 [M]**0.5 % (v/v)** (Sigma-Aldrich 9002-93-1)
- β-mercaptoethanol [M]**10 Milimolar (mM)** (add right before use) (Fisher 21985023)
- Roche cOmplete™ EDTA-free Protease Inhibitor Tablet (add right before use) (Roche 11873580001)

Equilibration buffer (prepare at least **250 mL**)

- HEPES [M]**50 Milimolar (mM)** pH**7.5** (Sigma-Aldrich 7365-45-9)
- NaCl [M]**300 Milimolar (mM)** (Sigma-Aldrich 7647-14-5)
- Imidazole [M]**20 Milimolar (mM)** (Sigma-Aldrich 288-32-4)

Column buffer 1 (prepare at least **250 mL**)

- HEPES [M]**50 Milimolar (mM)** pH**7.5** (Sigma-Aldrich 7365-45-9)
- NaCl [M]**300 Milimolar (mM)** (Sigma-Aldrich 7647-14-5)
- Imidazole [M]**50 Milimolar (mM)** (Sigma-Aldrich 288-32-4)

Column buffer 2 (prepare at least **250 mL**)

- HEPES [M]**50 Milimolar (mM)** pH**7.5** (Sigma-Aldrich 7365-45-9)
- NaCl [M]**300 Milimolar (mM)** (Sigma-Aldrich 7647-14-5)
- Imidazole [M]**70 Milimolar (mM)** (Sigma-Aldrich 288-32-4)

Elution buffer (prepare at least **250 mL**)

- HEPES [M]**50 Milimolar (mM)** pH**7.5** (Sigma-Aldrich 7365-45-9)
- NaCl [M]**300 Milimolar (mM)** (Sigma-Aldrich 7647-14-5)
- Imidazole [M]**250 Milimolar (mM)** (Sigma-Aldrich 288-32-4)

FPLC Buffer A (prepare at least **500 mL**)

- Tris-HCl [M]**50 Milimolar (mM)** pH**8.0** (Sigma-Aldrich 9210-OP)
- NaCl [M]**100 Milimolar (mM)** (Sigma-Aldrich 7647-14-5)
- DTT [M]**1 Milimolar (mM)** (add right before use) (Fisher R0861)

FPLC Buffer B (prepare at least **500 mL**)

- Tris-HCl [M]**50 Milimolar (mM)** pH**8.0** (Sigma-Aldrich 9210-OP)
- NaCl [M]**1 Molarity (M)** (Sigma-Aldrich 7647-14-5)
- DTT [M]**1 Milimolar (mM)** (add right before use) (Fisher R0861)

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Ni-NTA agarose (QIAGEN 30210)
HiPrep 26/10 Desalting column (Cytiva 17508701)
HiTrap Q HP column (Cytiva 29051325)
HiTrap SP HP column (Cytiva 17115201)
4x SDS loading buffer
10K Amicon Ultra-15 tube (Sigma-Aldrich UFC900308)
Glycerol [M]**80 % (v/v)** (Fisher 17904)

Samples for Quality Control

1 Taking samples throughout the purification process can help with quality control/troubleshooting (if something goes wrong) and is generally good practice for tracking the purification of your protein. You can take samples at any steps you want, but a general outline for samples to collect is the following.

1.1 Sample 1: Lysate from before sonication. Combine 30 µL of lysate with 10 µL of 4x SDS loading buffer.

1.2 Sample 2: Lysate from after sonication and centrifugation. Combine 60 µL of supernatant with 20 µL of 4x SDS loading buffer.

1.3 Sample 3: Pellet from after sonication and centrifugation. Resuspend a small amount (pipette tip to scoop) of pellet in ~100 µL of 1x SDS loading buffer.

1.4 Sample 4: Flow-through (unbound solution) from Ni-NTA His column using gravity flow. Combine 60 µL of flow-through and 20 µL of 4x SDS loading buffer.

1.5 Sample 5: Flow-through after buffer 1 wash. Combine 60 µL of flow-through and 20 µL of 4x SDS loading buffer.

1.6 Sample 6: Flow-through after buffer 2 wash. Combine 60 µL of flow-through and 20 µL of 4x SDS loading buffer.

1.7 Sample 7: Flow-through after elution buffer (this should have the protein). Combine 60 µL of flow-through and 20 µL of 4x SDS loading buffer.

1.8 Sample 8: Buffer-exchanged sample from HiPrep 26/10 desalting column. Combine 60 µL of sample and 20 µL of 4x SDS loading buffer.

1.9 Sample 9: Flow-through from HiPrep Q/SP HP column - first column volume during the FPLC buffer A wash. Combine 60 µL of flow-through and 20 µL of 4x SDS loading buffer.

1.10 Fraction samples: Samples from each fraction in the peak after HiPrep SP HP column elution. Combine 10 µL of fraction with 10 µL of 4x SDS loading buffer and 20 µL of Milli-Q water.

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Induction

2 Transform pA-Hia5 into T7 Express lysY competent *E. coli* cells for recombinant protein expression.

3 Grow 200 mL of starter culture in LB broth at 37ºC (shaking) with 50 mg/mL kanamycin and 34 mg/mL chloramphenicol to an OD_{600} of 0.6.

4 Dilute 200 mL culture into 2 L LB broth with 50 mg/mL kanamycin and grow at 37ºC (shaking) to an OD_{600} of 0.8 - 1.0.

5 Induce protein expression with a final concentration of 1 mM IPTG (e.g. 1 mL of 1 M IPTG into 1 L of culture) and grow for 4 hours at 20ºC (shaking).

6 Pellet cells at 5000 x g for 15 minutes at 4ºC.

7 Add 35 mL of lysis buffer (50 mM HEPES, pH 7.5; 300 mM NaCl; 10% glycerol; 0.5% Triton X-100) per 1 L of pelleted cell culture.

8 Freeze resuspended cells in liquid nitrogen and store at -80ºC.

Purification

9 On the day of purification, thaw cells (store on ice) and add 2x EDTA-free protease inhibitor tablet and 10 mM β-mercaptoethanol per 2 L of pelleted cell culture.

10 Lyse cells by probe sonication (Qsonica Q125) (6 pulses, 30s on, 1 min off at 200W).

11 Centrifuge lysed cells for 1 hour at 40,000 x g at 4ºC in 50 mL Oakridge tubes.

12 While lysed cells are pelleting, prepare Ni-NTA agarose by washing 2x with 30 mL of equilibration buffer (50 mM HEPES, pH 7.5; 300 mM NaCl; 20 mM imidazole) per 5 mL of Ni-NTA slurry. Prepare 5 mL of Ni-NTA slurry per 2 L of cells.

13 Combine cell lysate with washed Ni-NTA agarose and rotate 1 hour at 4ºC. It reduces agarose/sample loss to rotate it directly in the (sealed) gravity column that you will use in the next steps.

14 If mixture is not already in the gravity column, then pour it in now. Set up the column and collect flow-through.

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15 Wash with 40 mL of buffer 1 (50 mM HEPES, pH 7.5; 300 mM NaCl; 50 mM imidazole) and collect flow-through.

16 Wash with 30 mL of buffer 2 (50 mM HEPES, pH 7.5; 300 mM NaCl; 70 mM imidazole) and collect flow-through.

17 Elute with 30 mL of elution buffer (50 mM HEPES, pH 7.5; 300 mM NaCl; 250 mM imidazole) and collect flow-through (your protein should be here).

Note: The protein might crash out if you dialyze it overnight. Using a desalting column is highly recommended.

18 Filter eluted protein with a 0.2 µm filter.

19 Load filtered protein onto a HiPrep 26/10 Desalting column and buffer exchange into FPLC buffer A (50 mM Tris-HCl, pH 8.0; 100 mM NaCl; 1mM DTT).

20 Load buffer-exchanged sample onto tandem HiTrap Q HP and HiTrap SP HP columns. Wash both columns with 5x combined column volume (volume of Q + SP columns) of FPLC buffer A.

21 Remove the HiTrap Q HP column and elute from the HiTrap SP HP column using a linear gradient of 20x column volumes with increasing concentration of FPLC buffer B (50 mM Tris-HCl, pH 8.0; 1 M NaCl; 1 mM DTT). Save the HiTrap Q HP column for elution later, just in case protein is not in the HiTrap SP HP column.

22 Collect fractions and quantify using A280 absorbance. Verify presence of the protein in the collected fractions using an SDS-PAGE gel.

23 Concentrate peak fractions to final protein concentration > 5 µM using a 10K Amicon Ultra-15 tube. Supplement final concentrated sample with 10% glycerol (final concentration), aliquot it, freeze it with liquid nitrogen, and store it at -80ºC.

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