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Recombinant expression and purification of HIV-1 RT

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Reclone.org (The Reagent Collaboration Network)
Tech. support email: **protocols@recode.org**
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ABSTRACT

This protocol has been optimized for the recombinant expression of HIV-1 RT. The sequence of the plasmid encoding HIV-1 RT can be found on reclone.org.

The goal of this protocol was to eliminate the use of large volumes for dialysis and its fast buffer exchange into storage conditions.

MATERIALS TEXT

MATERIALS

- Sodium phosphate monobasic monohydrate **Sigma Aldrich Catalog #S9638**
- PMSF **Sigma Aldrich Catalog #P7626**
- Sodium phosphate dibasic **Sigma Aldrich Catalog #7558-79-4**
- DTT **Sigma Aldrich Catalog #D0632**
- Imidazole **Sigma Catalog #I5513**
- NaCl **Sigma Aldrich Catalog #53014**
- HisTrap FF Crude Column **Ge Healthcare Catalog #17528601**
- Glycerol **Sigma Aldrich Catalog #G5516**
- Dextrose **Sigma – Aldrich Catalog #D9434**
- Tween-20 **Sigma Aldrich Catalog #P9416**
- EDTA **Sigma Aldrich Catalog #ED2SS**
- Trizma Base **Contributed by users Catalog #93362**
- Lysozyme **Thermo Fisher Scientific Catalog #89833**
- Amicon Ultra-15 Centrifugal Filter Unit **Emd Millipore Catalog #UFC910024**

Buffer A, pH 8.0

[M] 50 millimolar (mM) NaPO4, pH 8.0

[M] 50 millimolar (mM) dextrose
[M] 300 millimolar (mM) NaCl
[M] 1 millimolar (mM) EDTA
[M] 0.1 % volume Tween-20
[M] 40 millimolar (mM) Imidazole, pH 8.0

Buffer B, pH 8.0

[M] 50 millimolar (mM) Tris-HCl, pH 8.0
[M] 300 millimolar (mM) NaCl
[M] 1 millimolar (mM) EDTA
[M] 0.1 % volume Tween-20
[M] 10 % volume Glycerol
[M] 40 millimolar (mM) Imidazole, pH 8.0

Buffer C, pH 8.0

[M] 50 millimolar (mM) Tris-HCl, pH 8.0
[M] 300 millimolar (mM) NaCl
[M] 1 millimolar (mM) EDTA
[M] 0.1 % volume Tween-20
[M] 10 % volume Glycerol
[M] 150 millimolar (mM) Imidazole, pH 8.0

Buffer D, pH 8.0

[M] 50 millimolar (mM) Tris-HCl, pH 8.0
[M] 300 millimolar (mM) NaCl
[M] 1 millimolar (mM) EDTA
[M] 0.1 % volume Tween-20

Storage Conditions

[M] 25 millimolar (mM) Tris-HCl, pH 8.0
[M] 150 millimolar (mM) NaCl
[M] 0.5 millimolar (mM) EDTA
[M] 5 millimolar (mM) DTT
[M] 0.05 % volume Tween-20
[M] 50 % volume Glycerol

DAY 1 – Plasmid transformation

1d

1 Transform 100 ng of plasmid containing HIV-1 RT into *E. coli* BL21(DE3) competent cells using either heat shock or electroporation. 2h

2 Spread transformed cells in LB Agar plates supplemented with [M] 0.05 mg/mL **Kan**. Grow plate overnight at 37 °C. 12h

DAY 2 – Preinoculum

1d

3 Select a single colony from the LB agar plate to prepare a preinoculum in 10 mL LB media supplemented with [M] 0.05 mg/mL **Kan**. Grow overnight at 250 rpm, 37°C. 1d

DAY 3 – Protein Overexpression

1d

4 Use the full volume of the preinoculum to inoculate 1 L of LB media supplemented with [M] 0.05 mg/mL **Kan** (1% inoculation). Grow at 250 rpm, 37°C until reaching an optical density at 600 nm (OD_{600}) = 0.8. 4h

5 Upon reaching OD_{600} = 0.8, add [M] 0.5 millimolar (mM) IPTG and incubate for 2h at 220 rpm, 37°C. 2h

DAY 4 – Protein Purification by IMAC

6h

6 Centrifuge the cell culture 4000 x g, 4°C, 00:20:00 .Then, resuspend the cell pellet in 50 mL of **Buffer A** freshly supplemented with [M] 0.5 millimolar (mM) PMSF and [M] 0.2 mg/mL lysozyme. 30m

7 Incubate the resuspended cells 80 rpm, Room temperature , 00:30:00. 30m

8 Sonicate on ice for 00:04:00 using cycles of 00:00:06 ON and 00:00:06 OFF at 40% amplitude (Qsonica Q125, 125W). 30m

9 Centrifuge the unclarified lysate 20000 x g, 4°C, 00:20:00 and collect the supernatant. You might want to collect a small sample for SDS-PAGE afterwards. 30m

10 On a **1 mL HisTrap column (Ge Healthcare)** preequilibrated with 10 column volumes (c.v.) (here, 10 mL) of **Buffer A**, load the supernant. Wash with 10 c.v. of **Buffer A.** Then, wash with 10 c.v. of **Buffer B**, and elute with 5 c.v. of **Buffer C**, collecting the eluted fractions every 0.5 mL in 1.5 ml tubes. 1h

11 To quickly pool the fractions containing the protein of interest, prepare a 96-well plate or 1.5 mL tubes with 40 µL of Bradford reagent and 160 µL of distilled water. Then, add 10 µL of each protein fraction and compare against a blank reference sample corresponding to 10 µL of **Buffer C**. You can determine your protein-containing fractions either by absorbance at 595 nm on a plate reader or visually by comparing the blue coloration of each fraction against the blank reference. Pool your fractions and collect a 10 µL sample for SDS-PAGE 5m

12 To decrease the imidazole concentration, perform a buffer exchange step with an Amicon Ultra-15 concentrator (Merck Millipore). Centrifuge 3000 x g, 10°C, 00:10:00, discard the flowthrough, add Buffer D to decrease the imidazole concentration and repeat this step, until the imidazole concentration reaches < 30 mM. 40m

13 Recover the concentrated protein and determine its concentration using the Bradford assay. Also, collect a 10 µL sample for SDS-PAGE. 5m

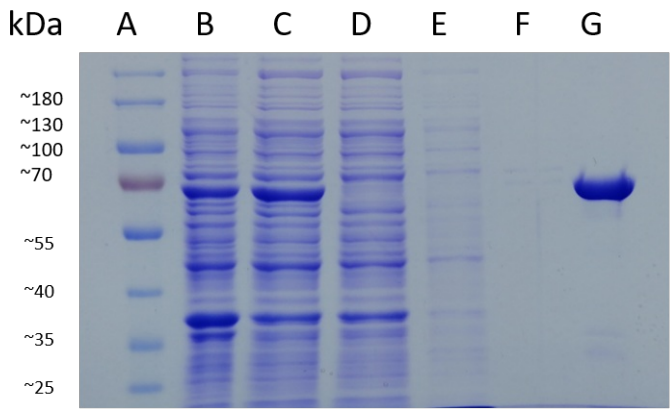
14 For storage, supplement your pooled fraction with [M] 10 millimolar (mM) DTT. Then, add glycerol up to [M] 50 % volume to reach **Storage Conditions.** Do consider that a final protein concentration of [M] 1 mg/mL is appropriate for subsequent experiments. 5m

15 Generate 200 µL aliquots of the enzyme and store it at -20 °C until required. 30m

IMAC SDS-PAGE Result 10m

16

Expected result



SDS PAGE for the recombinant expression and purification of HIV-1 RT in *E. coli* BL21 (DE3) using the open pTi vector (A) Protein marker; (B) Pellet (C) Clarified cell lysate; (D) Flowthrough; (E) First washing step; (F) Second washing step; (G) Pooled eluted fractions