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

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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

MATERIALS

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