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Expression and purification of recombinant MM4 reverse transcriptase (RT)

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Diana A Tapia-Sidas

ABSTRACT

Reverse transcriptases (RTs) are RNA-dependent DNA polymerases able of synthesizing DNA (complementary DNA or cDNA) from an RNA template. RTs are especially useful in RNA-based nucleic acid detection techniques. Due to its high catalytic activity and fidelity, one of the most widely used RTs in diagnostics and molecular biology is the RT from the Moloney Murine Leukemia Virus (MMLV). However, RT-MMLV is thermally unstable, so previous studies have produced a RT variant called RT-MM4 carrying mutations of positive charges in four amino acids (E286R/E302K/L435R/D524A). This protocol describes the optimized expression process, as well as the FPLC purification of RT-MM4 for use in isothermal amplification techniques, such as end-point colorimetric or real-time fluorometric RT-LAMP.

GUIDELINES

During the process of protein purification maintain all samples that contain the protein of interest in a cold environment to avoid protein degradation.

MATERIALS

Protocol status: Working

Created: Jun 08, 2022

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PROTOCOL integer ID: 64229

Keywords: Moloney Murine Leukemia Virus, thermostable reverse transcriptase, protein expression, protein purification, MM4 RT

Reagents:

- Chaperone Plasmid Set **Takara Bio USA, Inc. Catalog #3340**
-
Chemically Competent E. coli One Shot™ BL21(DE3) **Invitrogen - Thermo Fisher Catalog #C600003**
- [M] 100 ng/µL pET-MM4-RT plasmid Step 3.1
- Stock [M] 30 mg/mL chloramphenicol
- Stock [M] 100 mg/mL carbenicillin
- Stock [M] 1 Molarity (M) IPTG
- Stock [M] 0.5 Molarity (M) EDTA
- Stock [M] 1 Molarity (M) DTT
- Stock [M] 10 % (v/v) Triton X-100
- 250 µL of SOC medium Step 1.4
- 8 mL of TFBI solution Step 2.4
- 2.5 mL of TFBII solution Step 2.5
- Cryotubes with 500 µL of 30% v/v glycerol (sterilized)
- LB agar plates
- Tubes with 3 mL Luria-Bertani (LB) medium
- Tubes with 5 mL Luria-Bertani (LB) medium
- Tubes with 3 mL Terrific Broth (LB) medium
- Flasks with 20 mL LB medium
- Flasks with 50 mL LB medium
- Flasks with 50 mL TB medium
- Flask with 100 mL LB medium
- Flasks with 1 L LB medium
- 500 mL Lysis Buffer B (LB-B) Step 5.5/8.4
- 500 mL Elution Buffer-BI (EB-BI) Step 10.5
- 1 L Desalting Buffer-B (DB-B) Step 11.2
- 500 mL Elution Buffer-BII (EB-BII) Step 12.5
- 500 mL Storage Buffer-B (SB-B) Step 13.1
- HisTrap HP 5mL **Cytiva Catalog #17524801**
- HiPrep 26/10 Desalting Column **Cytiva Catalog #17508701**
- HiTrap SP XL 5mL **Cytiva Catalog #17516101**
-
Quick Start™ Bradford 1x Dye Reagent **BioRad Sciences Catalog #5000205**
-

Precision Plus Protein™ Unstained Protein Standards **Bio-rad Laboratories Catalog #1610363**

- Tricine-SDS-PAGE electrophoresis solutions (Step 6.2)
- 8% polyacrylamide gels for Tricine-SDS-PAGE (Step 6.2)

Equipments:

- Thermomixer

Equipment	
Thermomixer® R	NAME
Dry block heating and cooling shaker, 120 V, 60 Hz, 1/cs	TYPE
Eppendorf	BRAND
T3317	SKU

- Orbital shaker

Equipment	
MaxQ™ HP Incubated Tabletop Orbital Shaker	NAME
MaxQ™ HP, 120 V 60 Hz, 6,5 A o 230 V 50/60 Hz, 3,2 A	TYPE
Thermo Scientific	BRAND
SHKE420HP	SKU
https://www.thermofisher.com/order/catalog/product/SHKE420HP	LINK

- Floor model orbital shaker

Equipment

MaxQ™ HP Incubated and Refrigerated Console Shakers NAME

MaxQ™ 481 HP, 230 V, 50 Hz TYPE

Thermo Scientific BRAND

SHKE481HP SKU

https://www.thermofisher.com/order/catalog/product/SHKE481HP LINK

- Centrifuge

Equipment

Sorvall™ Legend™ XT/XF Centrifuge Series NAME

Thermo Scientific BRAND

75004541 SKU

https://www.thermofisher.com/order/catalog/product/75004541 LINK

- Ultrasonic Processor 130W

Equipment

Ultrasonic Processor NAME

130-Watt Ultrasonic Processor TYPE

Cole-Parmer BRAND

ML-04714-52 SKU

https://www.coleparmer.com/p/cole-parmer-130-watt-ultrasonic-processors/44347 LINK

- Ultrasonic Processor 750W

Equipment

750-Watt Ultrasonic Processor	NAME
CPX750	TYPE
Cole-Parmer	BRAND
ML-04711-60	SKU
https://www.coleparmer.com/p/cole-parmer-500-and-750-watt-ultrasonic-processors/16401	LINK

-Nanodrop

Equipment

NanoDrop™ One UV-Vis Spectrophotometer	NAME
spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU
https://www.thermofisher.com/order/catalog/product/ND-ONE-W	LINK
Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm	SPECIFICATIONS

- FPLC system

Equipment	
ÄKTA pure	NAME
Protein purification system	TYPE
Cytiva	BRAND
29046665	SKU
https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665	LINK

- Spectrophotometer UV/Vis
- Incubator (37°C)
- Water bath (60°C)
- Ultra Low–Temperature Freezer (-80°C)
- Freezer -20°C
- Refrigerator (4°C)
- Analytical balance

Other:
- Ice bath
- Microcentrifuge tubes
- Sterile 0.45 µm membrane filter
- 150 mL Superloop (Cytiva)
- Dialysis membrane
- Ultrafiltration tube (Amicon Ultra-15)

Equipment

Amicon Ultra-15	NAME
PLTK Ultracel-PL membrane, 15 ML - 30 kDa cutoff	TYPE
Millipore	BRAND
UFC903024	SKU
https://www.merckmillipore.com/MX/es/product/Amicon-Ultra-15-Centrifugal-Filter-Unit,MM_NF-UFC903024?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1	LINK

- Image Lab 6.1 Software (Bio-Rad)

BEFORE START INSTRUCTIONS

Ensure to have all the necessary materials and reagents already cleaned, sterilized and filter (in case of the purification solutions).

Preparation of RT expression cells

2d 12h 20m 53s

1 Transformation of chemically competent BL21 (DE3) cells with pKJE7 plasmid.

1.1 Add 1 µL of plasmidic DNA consisting of the pKJE7 plasmid from the Chaperone Plasmid Set **Takara Bio USA, Inc. Catalog #3340** to 50 µL of competent cells Chemically Competent E. coli One Shot™ BL21(DE3) **Invitrogen - Thermo Fisher Catalo #C600003** . Mix the cells gently and incubate On ice for 00:20:00 .

20m

1.2 Transfer the cells to a heat block at 42 °C and incubate for 00:00:53 .

53s

1.3 Transfer the cells immediately to an ice bath and incubate On ice for 00:05:00 . 5m

1.4 Add 250 µL of SOC medium at room temperature to the transformed cells and incubate at 225 rpm, 37°C, 01:00:00 . 1h

Note

SOC medium composition

	A	B
	Tryptone	2%
	Yeast extract	0.5%
	NaCl	10 mM
	KCl	2.5 mM
	MgCl2	10 mM
	MgSO4	10 mM
	Glucose	20 mM

Adjust to pH 7 and sterilize by filtration.

1.5 Plate 25 µL of transformed cells culture onto LB agar with the corresponding selective agent. Incubate the plates Overnight at 37 °C . 18h

Note

The pKJE7 plasmid requires [M] 30 µg/mL chloramphenicol as selective agent.

1.6 Select a single colony of transformed cells and inoculate in 3 mL Luria-Bertani (LB) medium supplemented with the selective antibiotic. Incubate 18h

Overnight at 180 rpm, 37°C .

1.7 Centrifugate the cell culture at 10000 x g, 4°C, 00:05:00 . Remove the supernatant and resuspend the pellet in 500 µL LB medium . 5m

1.8 Add 500 µL of 30% v/v glycerol , mix by pipetting up and down and store at -80 °C . 5m

2 Preparation of chemically competent BL21 (DE3) cells harboring pKJE7 plasmid.

2.1 Take BL21(DE3) cells harboring pKJE7 plasmid from a frozen glycerol stock using a bacterial inoculating loop and inoculate 3 mL LB liquid medium with [M] 30 µg/mL of chloramphenicol . Incubate Overnight at 180 rpm, 37°C . 18h

2.2 Inoculate 1 mL overnight culture in 100 mL LB medium with [M] 30 µg/mL chloramphenicol and incubate at 180 rpm, 37°C, 03:00:00 . 3h

> Note
>
> Monitor the cell growth by measuring the optical density **(OD) at 600 nm** and remove the cells from incubation when the OD reaches **0.3 to 0.4.**

2.3 Chill the cell culture On ice for 00:10:00 and centrifugate the cells at 4000 x g, 4°C, 00:10:00 . 20m

2.4 Gently resuspend the cell pellet in 8 mL of TFBI solution pre-coolded and incubate On ice for 00:45:00 . Centrifugate the cells at 4000 x g, 4°C, 00:05:00 . 50m

Note

TFBI medium composition

	A	B
	Potassium acetate	30 mM
	Rubidium chloride	100 mM
	Calcium chloride	10 mM
	Manganese chloride	50 mM
	Glycerol	15% v/v

Adjust to pH 5.8 with 1M acetic acid and sterilize by filtration.

2.5 Gently resuspend the cell pellet in 2.5 mL of TFBII solution pre-coolded and incubate On ice for 00:05:00. 5m

Note

TFBII medium composition

	A	B
	MOPS	10 mM
	Rubidium chloride	10 mM
	Calcium chloride	75 mM
	Glycerol	15% v/v

Adjust to pH 6.5 with 1M sodium hydroxide and sterilize by filtration.

2.6 Prepare aliquots of 50 µL of competent cells using microcentrifuge tubes previously 30m

chilled on an ice bath. Place the aliquots on dry ice until frozen and store at -80 °C.

3 **Transformation of chemically competent BL21 (DE3)/pKJE7 cells with the pET-MM4-RT plasmid, the expression vector for the quadruple mutant (E286R/E302K/L435R/D524A, designated as MM4) of the reverse transcriptase (RT) from Moloney Murine Leukemia Virus (MMLV).**

CITATION

Yasukawa K, Mizuno M, Konishi A, Inouye K (2010). Increase in thermal stability of Moloney murine leukaemia virus reverse transcriptase by site-directed mutagenesis.. Journal of biotechnology.

LINK

https://doi.org/10.1016/j.jbiotec.2010.09.961

3.1 Add 1 µL of plasmidic DNA of [M] 100 ng/µL pET-MM4-RT expression vector to 50 µL of competent cells BL21 (DE3)/pKJE7. For the transformation procedure go to step #1.

Note

The pET-MM4-RT plasmid requires [M] 100 µg/mL carbenicillin as selective agent and the pKJE7 plasmid requires [M] 30 µg/mL chloramphenicol. Use LB medium supplemented with both antibiotics as selective media.

Small-scale screening cultures

1d 14h 16m 45s

4 **Preparation of bacterial cultures for RT expression**.

4.1 Inoculate 5 µL glycerol stock of **BL21(DE3)/pKJE7/pET-MM4-RT** or **BL21(DE3)/pKJE7** cells in 5 mL culture medium (LB or TB) supplemented with selection agent(s). Incubate Overnight at 200 rpm, 37°C. 18h

4.2 For each treatment, inoculate 500 µL overnight culture in 50 mL culture medium with [M] 100 µg/mL carbenicillin. Use LB or TB according to the medium use for the overnight culture. Incubate 200 rpm, 37°C, 03:00:00. 3h

> Note
>
> Monitor the cell growth by measuring the optical density **(OD) at 600 nm** and remove the cells from incubation when the OD reaches **0.6.**

4.3 Once the culture reaches an OD600 of 0.6, incubate the cell cultures On ice for 00:20:00 before adding the inducer (IPTG). 20m

5 Small-scale RT expression under different induction conditions.

5.1 Induce the expression of the RT under different conditions. Each treatment should be evaluated in triplicate. For example: 30m

	Strain	[IPTG]	Temperature	Medium
	BL21(DE3)/pKJE7*	0.5 mM	16°C	LB
	BL21(DE3)/pKJE7*	0.5 mM	37°C	LB
	BL21(DE3)/pKJE7*	0.5 mM	16°C	TB
	BL21(DE3)/pKJE7/pET-MM4-RT	0 mM	16°C	LB
	BL21(DE3)/pKJE7/pET-MM4-RT	0.1 mM	16°C	LB
	BL21(DE3)/pKJE7/pET-MM4-RT	0.5 mM	16°C	LB
	BL21(DE3)/pKJE7/pET-MM4-RT	1.0 mM	16°C	LB
	BL21(DE3)/pKJE7/pET-MM4-RT	0 mM	37°C	LB
	BL21(DE3)/pKJE7/pET-MM4-RT	0.5 mM	37°C	LB

	Strain	[IPTG]	Temperature	Medium
	BL21(DE3)/pKJE7/pET-MM4-RT	0.5 mM	16°C	TB

* BL21(DE3)pKJE7 strain is used as negative expression control.

5.2 Incubate at 16 °C or 37 °C according to each treatment at 180 rpm for 16:00:00 . 16h

5.3 Centrifugate the cell cultures at 6000 x g, 4°C, 00:10:00 . Discard the supernatant, remove all the liquid and leave the cell pellet as dry as posible. 10m

5.4 Weigh the centrifugation tube with the cell pellet (total weight). 10m

> Note
>
> Weigh the empty tube prior centrifugation and subtract it to the total weight to calculate the weight of the cell pellet and hence the biomass produced.

5.5 Resuspend the cell pellet in 5 mL lysis buffer B (LB-B) (pre-cooled). 5m

Note

Lysis buffer B composition (LB-B)

	A	B
	NaH2PO4/Na2HPO4 pH7.8	50 mM
	NaCl	300 mM
	DTT	2.5 mM
	Imidazole	10 mM
	Glycerol	5% v/v
	PMSF	3 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.8. Store at 4°C.

5.6 Disrupt cells by ultrasonication at an amplitude of 40%. Apply five cycles of 00:00:15 on 3m 45s and 00:00:30 off.

Note

Place the tubes On ice while processing.

Equipment

Ultrasonic Processor	NAME
130-Watt Ultrasonic Processor	TYPE
Cole-Parmer	BRAND
ML-04714-52	SKU
https://www.coleparmer.com/p/cole-parmer-130-watt-ultrasonic-processors/44347	LINK

5.7 Centrifugate at 6000 x g, 4°C, 00:15:00. Recover the supernatant (soluble protein fraction) and discard the pellet. 15m

6 Analysis of RT expression.

6.1 Measure total protein concentration by measuring absorbance at 280 nm in a NanoDrop spectrophotometer. 3m

Equipment

NanoDrop™ One UV-Vis Spectrophotometer	NAME
spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU
https://www.thermofisher.com/order/catalog/product/ND-ONE-W	LINK
Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm	SPECIFICATIONS

6.2 Analyze all supernatant samples by Tricine-SDS-PAGE electrophoresis through a 8% polyacrylamide gel. Load 100 µg protein sample per well. 4h

CITATION

Hermann Schägger (2006). Tricine-SDS-PAGE. Nature Protocols.
LINK
10.1038/nprot.2006.4

6.3 Select the best conditions for protein expression according to the results analysis (biomass, total protein production and electrophoretic profile).

Large-scale production of RT

1d 14h 24m 20s

7 **Expression of recombinant RT.**

7.1 Inoculate 10 µL glycerol stock of **BL21(DE3)/pKJE7/pET-MM4-RT** expression cells in 20 mL LB medium supplemented witt [M] 100 µg/mL carbenicillin and [M] 30 µg/mL chloramphenicol. Incubate Overnight at 180 rpm, 37°C. 18h

7.2 Inoculate 10 mL overnight culture in 1 L LB medium with [M] 100 µg/mL carbenicillin and [M] 30 µg/mL chloramphenicol. Incubate 200-220 rpm, 37°C, 03:00:00. 3h

7.3 Place the inoculum On ice for 00:30:00 and then add [M] 0.5 millimolar (mM) IPTG for induction. 30m

Note

Do not add any additional inducers. For expression of the chaperones contained in pKJE7 plasmid, the basal expression is enough to promote correct RT enzyme folding.

7.4 Incubate at 180 rpm, 16°C, 16:00:00 for recombinant protein expression. 16h

8 Soluble protein fraction recovery

8.1 Centrifugate at 6000 x g, 4°C, 00:12:00 to harvest cells. Discard the supernatant ensuring to remove all the liquid and leave the cell pellet as dry as posible. 12m

8.2 Weigh the centrifugation tube with the cell pellet (total weight). 3m

Note

Weigh the empty tube prior centrifugation and subtract it to the total weight to calculate the weight of the cell pellet and hence the biomass produced.

8.3 Store the cell pellet at -80 °C until use (just in case that the purification step is not performed immediately after expression).

8.4 Resuspend the cell pellet in 50 mL lysis buffer B (LB-B) (pre-cooled). If neccesary, defroze the cell pellet in an ice bath before adding the lysis buffer. 5m

Note

Lysis buffer B composition (LB-B)

	A	B
	NaH2PO4/Na2HPO4 pH7.8	50 mM
	NaCl	300 mM
	DTT	2.5 mM
	Imidazole	10 mM
	Glycerol	5% v/v
	PMSF	3 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.8. Store at 4°C.

8.5 Disrupt cells by ultrasonication with an ultrasonic processor at an amplitude of 40% applying pulses of 00:00:10 of ultrasonication and 00:00:10 of pause during 00:04:00. 4m 20s

Note

Place the sample On ice and keep it cold while processing.

Equipment	
750-Watt Ultrasonic Processor	NAME
CPX750	TYPE
Cole-Parmer	BRAND
ML-04711-60	SKU
https://www.coleparmer.com/p/cole-parmer-500-and-750-watt-ultrasonic-processors/16401	LINK

8.6 Centrifugate at 11000 x g, 4°C, 00:30:00. Recover the supernatant (soluble protein fraction) and discard the pellet. 30m

> **Note**
>
> Place the supernatant in an ice bath or store at 4°C until use.

Purification of recombinant RT by FPLC

9 Sample preparation.

> **Note**
>
> Keep all protein samples On ice during the purification process to avoid protein degradation.

9.1 Filter the supernatant (soluble protein fraction) through a 0.45 µm membrane.

9.2 Load the soluble protein fraction onto a 150 mL Superloop (Cytiva). Store at 4 °C until use.

10 Immobilized metal affinity chromatography (Ni^{2+}-IMAC).

10.1 Connect a HisTrap HP 5mL **Cytiva Catalog #17524801** to a FPLC system.

Equipment	
ÄKTA pure	NAME
Protein purification system	TYPE
Cytiva	BRAND
29046665	SKU
https://www.cytivalifesciences.com/en/us/support/products/akta-pure-150-l-29046665	LINK

10.2 Equilibrate the column with 8 column volumes (CV) of lysis buffer B (LB-B) at a flow of 2.5 mL/min.

10.3 Connect the Superloop charged with the protein fraction and load the sample onto the column at a flow of
2.5 mL/min.

10.4 Wash the column with 10 CV of LB-B at a flow of 2.5 mL/min

10.5 Wash the column with 10 CV of 2% elution buffer-BI (EB-BI) at a flow of 2.5 mL/min.

Note

Elution buffer-BI composition (EB-BI).

	A	B
	NaH2PO4/Na2HPO4 pH7.8	50 mM
	NaCl	300 mM
	DTT	2.5 mM
	Imidazole	500 mM
	Glycerol	5% v/v
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.8. Store at 4°C.

10.6 Elute the proteins by passing 5 CV of 100% EB-BI through the column using a flow of 2.5 mL/min.

10.7 Immediatly after elution add [M] 2 millimolar (mM) EDTA and [M] 2.5 millimolar (mM) DTT to the eluted fractions.

10.8 Analyze all collected fractions by Tricine-SDS-PAGE electrophoresis through a 8% polyacrylamide gel. Load 10 µL protein sample per well.

10.9 Pool all elution fractions carrying the recombinant RT protein. Store at 4 °C until use.

11 Desalting step.

11.1 Connect a HiPrep 26/10 Desalting Column **Cytiva Catalog #17508701** to the FPLC system.

11.2 Wash the column with 2.5 CV of Mili-Q water. Then, equilibrate the column with 2 CV of desalting buffer-B (DB-B). For both steps use a flow of 10 mL/min.

Note

Desalting buffer-B composition (DB-B).

	A	B
	HEPES pH 7.5	50 mM
	NaCl	40 mM
	EDTA	2 mM
	DTT	5 mM
	Glycerol	5% v/v
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

11.3 Load the sample onto the column at a flow of 5 mL/min.

11.4 Wash the column with 2 CV of DB-B for protein elution at a flow of 10 mL/min.

11.5 Analyze all collected fractions by qualitative Bradford assay using the Quick Start™ Bradford 1x Dye Reagent **BioRad Sciences Catalog #5000205**. Pool the fractions with higher protein concentration.

11.6 Load the pool of desalted fractions onto a 150 mL Superloop (Cytiva). Store at 4 °C until use.

12 Cation exchange chromatography (CEC).

12.1 Connect a HiTrap SP XL 5mL **Cytiva Catalog #17516101** to the FPLC system.

12.2 Equilibrate the column with 10 CV of DB-B at a flow of 2 mL/min.

12.3 Connect the Superloop charged with the protein fraction and load the sample onto the column at a flow of 2 mL/min.

12.4 Wash the column with 5 CV of DB-B at a flow of 2 mL/min.

12.5 Elute proteins by washing the column with a linear gradient of 10 CV of elution buffer-BII (EB-BII). Use a flow of
2 mL/min.

Note

Elution buffer-BII composition (EB-BII).

	A	B
	HEPES pH 7.5	50 mM
	NaCl	1 M
	EDTA	2 mM
	DTT	5 mM
	Glycerol	5% v/v
	PMSF	1 mM

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

12.6 Analyze all collected fractions by Tricine-SDS-PAGE electrophoresis through a 8% polyacrylamide gel. Load 10 µL protein sample per well.

12.7 Pool all elution fractions carrying the recombinant RT protein. Store at 4 °C until use.

13 Purified RT enzyme concentration and formulation.

13.1 Load the purified RT enzyme pool onto a dialysis membrane (pre-hydrated). Place the membrane into a beaker with precooled storage buffer-B (SB-B) at a ratio 1:50 (v/v).

Note

Storage buffer-B composition (SB-B).

	A	B
	Tris-HCl pH 7.5	50 mM
	NaCl	150 mM
	EDTA	0.1 mM
	DTT	1 mM
	Glycerol	50% v/v

Prepare the buffer with Milli-Q water and adjust to pH 7.5. Store at 4°C.

13.2 Dialyze Overnight at 4 °C with slow agitation.

13.3 Recover the dialized protein, load it onto an **Amicon Ultra-15ML - 30 kDa cutoff centrifugal filter.** Concentrate until a concentration equal or higher than [M] 1 mg/mL.

Equipment

Amicon Ultra-15	NAME
PLTK Ultracel-PL membrane, 15 ML - 30 kDa cutoff	TYPE
Millipore	BRAND
UFC903024	SKU
https://www.merckmillipore.com/MX/es/product/Amicon-Ultra-15-Centrifugal-Filter-Unit,MM_NF-UFC903024?ReferrerURL=https%3A%2F%2Fwww.bing.com%2F&bd=1	LINK

Note

Monitor protein concentration measuring absorbance at 280 nm using a NanoDrop spectrophotometer.

Equipment

NanoDrop™ One UV-Vis Spectrophotometer	NAME
spectrophotometer	TYPE
Thermo Scientific	BRAND
ND-ONE-W	SKU
https://www.thermofisher.com/order/catalog/product/ND-ONE-W	LINK
Sample Volume (Metric): Minimum 1µL; Spectral Bandwidth: ≤1.8 nm (FWHM at Hg 254 nm); System Requirements: Windows™ 8.1 and 10, 64 bit; Voltage: 12 V (DC); Wavelength Range: 190–850 nm	SPECIFICATIONS

13.4 Prepare aliquots of 50 µL of concentrated RT enzyme .

13.5 Add [M] 0.05 % (v/v) tergitol NP-40 to the enzyme aliquots and store at -20 °C .

13.6 Determine final protein concentration by measuring absorbance at 280 nm in a NanoDrop spectrophotometer.

13.7 Analyze the final RT enzyme formulation by Tricine-SDS-PAGE electrophoresis through a 8% polyacrylamide gel. Load 3 µL protein sample per well. Load 3 µL protein ladder Precision Plus Protein™ Unstained Protein Standards **Bio-rad Laboratories Catalog #1610363** .

13.8 Analyze the electrophoresis gel by densitometry using the **Image Lab 6.1 Software (Bio-Rad)**. Determine protein concentration for each RT enzyme aliquot analyzed using the protein ladder as weight standard.

Note

The protein ladder Precision Plus Protein™ Unstained Protein Standards **Bio-rad Laboratories Catalog #1610363** includes three reference bands: The 50 KDa with 750 ng, the 20 KDa and 100 KDa bands with 150 ng each per each 10 µL of the protein ladder mix.