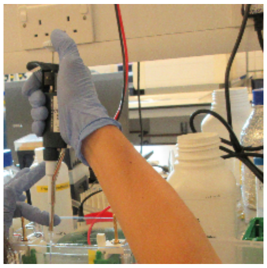
Jun 30, 2020



Purification of 2,3-bisphosphate-dependent phosphoglycerate mutase (dPGM)

In 1 collection

DOI
dx.doi.org/10.17504/protocols.io.bgawjsfe

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DOI: dx.doi.org/10.17504/protocols.io.bgawjsfe

Protocol Citation: Cristina Rodrigues Gabriel Sales, Anabela Silva, Elizabete Carmo-Silva 2020. Purification of 2,3-bisphosphate-dependent phosphoglycerate mutase (dPGM). **protocols.io https://dx.doi.org/10.17504/protocols.io.bgawjsfe**

Manuscript citation:
Sales CRG, Silva AB, Carmo-Silva E. 2020. Measuring Rubisco activity: challenges and opportunities of NADH-linked microtiter plate-based and 14C-based assays. Journal of Experimental Botany, https://doi.org/10.1093/jxb/eraa289

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

Created: May 12, 2020

Last Modified: June 30, 2020

Protocol Integer ID: 36918

Keywords: Protein purification, E. coli

Abstract

This protocol describes the purification of dPGM based on Fraser et al. (1999) and Scales et al. (2014) with modifications. Some steps of the purification protocol were also informed by van de Loo & Salvucci (1996), Schmidt & Skerra (2007), and White & Fothergill-Gilmore (1992).

Guidelines

1. Check the "Materials" tab for a list of all the chemicals used in this protocol.
2. In the "Steps" tab there is a brief description of the materials and equipment necessary for the protocol execution.
3. The references cited are at the end of the "Materials" tab.

Note

The figures uploaded in this protocol, explaining about the columns, are from iba lifesciences (https://www.iba-lifesciences.com/isotope/2/2-1002-100-Manual_Strep-Tactin-Purification.pdf)

Materials

MATERIALS

- Sodium hydroxide (NaOH) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**S5881
- 2-Mercaptoethanol **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**M6250
- Phenylmethanesulfonyl fluoride (PMSF) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P7626
- Tryptone **Neogen** **Catalog #**MC005
- Yeast extract **Neogen** **Catalog #**MC001
- Carbenicillin **Melford** **Catalog #**C0109
- Isopropyl-β-D-thiogalactopyranoside (IPTG) **Fisher Scientific** **Catalog #**10356553
- Glycerol **Alfa Aesar** **Catalog #**36646
- Potassium phosphate monobasic **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**P9791
- Sodium chloride (NaCl) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**31434-M
- Tris base **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**T1503
- Hydrochloric acid (HCl) **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**H1758
- 10x Buffer R; Strep-Tactin® Regeneration Buffer with HABA **iba** **Catalog #**2-1002-100
- Leupeptin hemisulfate **AG Scientific** **Catalog #**L-1165
- Sephadex® G-25 fine **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**G2580
- Strep-Tactin Sepharose 50% suspension **iba** **Catalog #**2-1201-010
- Ammonium sulfate **Merck MilliporeSigma (Sigma-Aldrich)** **Catalog #**A4418
- Desthiobiotin **iba** **Catalog #**2-1000-002
- Ethanol absolute 99.8 % **Fisher Scientific** **Catalog #**10437341

CITATION

IBA Life Sciences (2020). Expression and purification of proteins using Strep-Tactin. A comprehensive manual.

LINK

https://www.iba-lifesciences.com/isotope/2/2-1002-100-Manual_Strep-Tactin-Purification.pdf

CITATION

Fraser HI, Kvaratskhelia M, White MF (1999). The two analogous phosphoglycerate mutases of Escherichia coli. FEBS Letters 455: 344-348.

LINK

10.1016/s0014-5793(99)00910-2

CITATION

van de Loo FJ, Salvucci ME (1996). Activation of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) involves Rubisco activase Trp16. Biochemistry 35: 8143-8148.

LINK

10.1021/bi9604901

CITATION

Scales JC, Parry MA, Salvucci ME (2014). A non-radioactive method for measuring Rubisco activase activity in the presence of variable ATP: ADP ratios, including modifications for measuring the activity and activation state of Rubisco. Photosynthesis Research 119: 355-365.

LINK

https://doi.org/10.1007/s11120-013-9964-5

CITATION

Schmidt TGM, Skerra A (2007). The Strep-tag system for one-step purification and high-affinity detection or capturing of proteins. Nature Protocols 2: 1528-1535.

LINK

https://doi.org/10.1038/nprot.2007.209

CITATION

White MF, Fothergill-Gilmore LA (1992). Development of a mutagenesis, expression and purification system for yeast phosphoglycerate mutase. Journal of Biochemistry 207: 709-714.

LINK

10.1111/j.1432-1033.1992.tb17099.x

Safety warnings

Before using the protocol always check the Safety Data Sheet (SDS) for each chemical.

Before start

MATERIAL & EQUIPMENTS (for list of chemicals check "Materials" tab)

- *E. coli* cells stored at -80°C as frozen glycerol stock: BL21Star(DE3)pLysS *E. coli* transformed with dPGM cDNA cloned into pET23a
- Shaking incubator(s) for 30°C and 37°C
- Vortex
- Microcentrifuge
- Bench top centrifuge
- Plate reader & LVis plate
- UV-Vis spectrophotometer
- Sonifier with standard tip

REAGENTS & SOLUTIONS

1 REAGENTS & SOLUTIONS TO PREPARE BEFOREHAND

1.1 LB medium

[M] 10 mg/mL Tryptone

[M] 5 mg/mL Yeast extract

- Add ultrapure H_2O and mix until dissolved; check if pH 7 and adjust with NaOH if required.
- Top volume up to the final volume with ultrapure H_2O; autoclave.

Note

Add [M] 10 mg/mL agar if making solid LB agar.

Note

To pour LB agar plates:

- Melt agar in the microwave if required (loosen the lid).
- Place into a water bath at 50 °C and allow agar to equilibrate to this temperature (at least 00:30:00).
- In the flow hood, add [M] 100 ng/ml carbenicillin and mix gently.
- Poor approximately 25 mL into each plate.
- Leave with lids off to set for approximately 00:25:00 .
- Store plates for up to 2-3 weeks in a sealed bag in the fridge (no parafilm).

Safety information

CAUTION – always watch the microwave when melting agar and never leave the area. The agar will become super-heated and can boil over very easily. Wear protective gloves.
CAUTION – never place anything cold (e.g. magnetic stirrer) into super-heated molten agar.

1.2 [M] 100 mg/mL Carbenicillin

- Dissolve in ultrapure H_2O; filter through a 0.25 µm sterile syringe filter.
  4 °C (storage)

1.3 [M] 0.1 Molarity (M) IPTG

- Dissolve in ultrapure H_2O; dispense in aliquots. -20 °C (storage)

1.4 Glycerol
Room temperature (storage)

1.5 **Buffer A**
[M] 0.1 Molarity (M) Potassium phosphate
[M] 75 millimolar (mM) NaCl

- Dissolve in ultrapure H_2O; adjust to pH 8 with NaOH; filter. 4 °C (storage)

1.6 **Buffer W (Washing buffer)**
[M] 100 millimolar (mM) Tris base
[M] 150 millimolar (mM) NaCl

- Dissolve in ultrapure H_2O; adjust to pH 8 with HCl; filter. 4 °C (storage)

1.7 **Buffer F (Final buffer)**
[M] 60 millimolar (mM) Tris base

- Dissolve in ultrapure H_2O; adjust to pH 7.9 with HCl; filter. 4 °C (storage)

1.8 **10x Buffer R**, Strep-Tactin Regeneration Buffer with HABA
4 °C (storage)

1.9 [M] 10 millimolar (mM) Leupeptin hemisulfate

- To the bottle received from AG Scientific (0.025 g) add 5.25 mL ethanol.
- Dispense in aliquots. -20 °C (storage)

1.10 2-Mercaptoethanol
Room temperature (storage)

1.11 [M] 100 millimolar (mM) PMSF

- Dissolve in ethanol. 4 °C (storage)

1.12 Sephadex G-25 fine

- Place 5 g of resin in **buffer A** (from step 1.5) and allow to swell for at least 03:00:00 at Room temperature
- Fill the glass column with 20 mL of bed volume and equilibrate with **buffer A** (3 times column volume).

Note

1 g swell to 4-6 mL.

1.13 Strep-Tactin Sepharose 50% suspension

- Bed volume of 20 mL . 4 °C (storage)
- Fill the glass column with the resin [it comes in 50% suspension in **buffer W** (from step 1.6), so it is already in the buffer that will be used later for purification (check below)].
- Equilibrate the column with 2 times column bed volumes of **buffer W**.

Note

Always work at 4 °C . If it is not possible to perform chromatography at 4 °C and column needs to be transferred to Room temperature air bubbles may form since cold storage buffer is able to take up more gas than buffers at Room temperature . Therefore, it is recommended to equilibrate the columns immediately after exposure to higher temperatures with buffer that is equilibrated at such temperature.

1.14 [M] 4.5 Molarity (M) Ammonium sulfate

- Add the powder in ultrapure H_2O; warm up with mixing to dissolve (mild heat).
- Adjusted to pH 7 with NH_4OH; let precipitate. 4 °C (storage)

1.15 Desthiobiotin

🌡 4 °C (storage)

2 SOLUTIONS TO PREPARE JUST BEFORE USE

- Prepared with reagents/solutions described in step 1.

2.1 Lysis buffer

Component	Stock	10 mL
Buffer A	(1x)	10 mL
10 mM 2-mercaptoethanol	14.26 M	7 µL
10 µM leupeptin hemisulfate	10 mM	10 µL
1 mM PMSF	0.1 M	100 µL

2.2 Complete Buffer W

Component	Stock	10 mL
Buffer W	(1x)	10 mL
10 mM 2-mercaptoethanol	14.26 M	7 µL
10 µM leupeptin hemisulfate	10 mM	10 µL
1 mM PMSF	0.1 M	100 µL

2.3 Buffer E (Elution buffer)

Component	Stock	10 mL
Buffer W	(1x)	10 mL
10 mM 2-mercaptoethanol	14.26 M	7 µL
10 µM leupeptin hemisulfate	10 mM	10 µL
1 mM PMSF	0.1 M	100 µL
5 mM desthiobiotin		0.011 g

2.4 Buffer R (Regeneration buffer)

Component	Stock	100 mL
Buffer R	(10x)	10 mL
Ultrapure H_2O		90 mL

PROCEDURE

3 CELL GROWTH & COLLECTION

Day 1

1. Inoculate the cultures with a small volume of cells using a sterile tip onto the plate containing LB medium with [M] 100 ng/ml carbenicillin.

2. Incubate plates upside down Overnight (approx. 9h) at 37 °C .

Note

Transformed *E. coli* cells stored as frozen glycerol stocks are used as inoculums for starter cultures. Keep glycerol stock On ice .

Day 2

3. Add [M] 100 ng/ml carbenicillin into 10 mL LB in a 50 mL Falcon tube.

4. Pick a single colony from the plate and add into the LB.

5. Shake Overnight (approx. 9h) at 37 °C .

Note

For large cultures do the above procedure in 4 Falcon tubes to get 40 mL of culture.

Day 3

6. Prepare a glycerol stock of the culture (for long term storage): combine 300 µL culture + 300 µL 30% glycerol in a 2 mL screw-cap tube, mix gently by inversion.

-80 °C (storage)

4 **INDUCTION**

Day 3

7. Dilute 1:50 in 1 L Erlenmeyer flask (20 mL of culture in a final volume of 1 L).

8. Grow cells at 37 °C under continuous stirring at 225 rpm.

9. Check optical density at 600 nm after 02:00:00 incubation, and keep checking regularly until the cells reach an optical density of approx. 0.8 at 600 nm (for the large culture it took ~ 04:00:00).

10. Remove 1 mL aliquot before adding IPTG for SDS-Page of uninduced and induced expression. Centrifuge, discard supernatant and keep at -20 °C for later.

11. Induce dPGM expression by adding [M] 0.1 Molarity (M) IPTG to the cell culture (1 mL of [M] 0.1 Molarity (M) IPTG in 1 L).

12. Maintain the culture at 29 °C Overnight under continuous stirring.

Note

Tests were done at 37 °C and 29 °C . Both had good results but at 29 °C more protein was produced.

Day 4

13. Transfer 400 mL culture to two 500 mL centrifuge bottles on ice.

14. Collect the cells by centrifuging at 4000 rpm, 4°C, 00:10:00 . Discard supernatant. Repeat using the same 500 mL bottles until the entire culture content is collected in the two bottles.

15. Suspend the accumulated cell pellets in about 20 mL of **buffer A**. Pool the cell suspension and distribute into 2× 50 mL screw cap tubes (On ice). Rinse the 500 mL bottles with about 5 mL of **buffer A** and add in the 50 mL tubes as well.

16. Centrifuge at 5000 x g, 4°C, 00:10:00 Discard supernatant and dry the tubes as much as possible. The wet cell pellets are stored at -80 °C until extraction.

5 **LYSIS**

Day 5 (long day)

17. Thaw pellets On ice (freeze-thaw cycle).

Note

Transfer tube with pellets to -20 °C in the evening before. On ice , keep pellet upside down, not directly touching ice to thaw faster.

18. Suspend the cell pellets to about [M] 5 mg/g of wet cell pellet weight to **lysis buffer**.

Note

E.g. if pellet weighs 2.694 g, add 13.47 mL buffer.

19. Vortex and keep On ice .
20. Transfer the cells to a beaker. Rupture the cells and DNA by sonicating the suspension until solution becomes easy to pipette. Insert sonicator half way into the solution and apply 3 × 45s bursts, maintaining the beaker always On ice . Pause for 30s between each burst.
21. Centrifuge at 26000 rpm, 4°C, 00:20:00 . Keep supernatant (and pellet for SDS-Page checking).
22. Analyse the expression of dPGM as soluble polypeptides in the uninduced and induced samples by SDS-PAGE (Appendix 2).

Note

It is useful to verify the expression of dPGM as insoluble and soluble polypeptides in the uninduced and induced samples by SDS-PAGE. It is expected that the protein will be in the soluble fraction but occasionally it may be in the insoluble fraction (see Appendix 2 for SDS-PAGE).

6 PRECIPITATION

23. Measure the volume of supernatant and add saturated ammonium sulfate to [M] 80 % (v/v)

Note

Vol saturated ammonium sulfate for 80% saturation = (Vol supernatant / 0.2) - Vol supernatant

24. Add the saturated ammonium sulfate drop-wise to the supernatant while stirring continuously using a separatory funnel in a ring stand. Stir for 00:30:00 .

25. Collect precipitated material by centrifugation at 20000 x g, 4°C, 00:13:00 (slow brake).

7 RESUSPENSION

26. Suspend pellets in a total volume of 3 mL of **buffer A** On ice , mixing well using a 1 mL pipette.

Note

If more than one tube, resuspend one and do the resuspension of the next one with the content of the first one, to reduce the volume produced. If needed, add more buffer.

27. Centrifuge at 20000 x g, 4°C, 00:20:00 to clean any remaining debris and be easier to flow through Sephadex G-25 fine column

8 DESALTING

28. After equilibrating Sephadex G-25 fine column with **buffer A** (2 x column volumes) and with **lysis buffer** (1 x column volume), load the sample and elute with **lysis buffer**, taking aliquots and checking the amount of protein with Bradford reagent (monitor the development of blue colour by eye for a qualitative assessment, not necessary to read absorbance).

29. Pool samples with highest protein content.

Note

Start adding 5 mL of **buffer A** and collecting the aliquots. Check TSP “by eye” and when it gets blue reduce to 2 mL elutions, until the aliquot has low protein content. The objective is to collect as much protein as possible without diluting the sample more than is necessary.

30. Once desalting of the sample is complete, wash column with 2 column volumes of [M] 0.2 Molarity (M) NaOH, rinse with water, and re-equilibrate with 2-3 column volumes of **buffer A**.

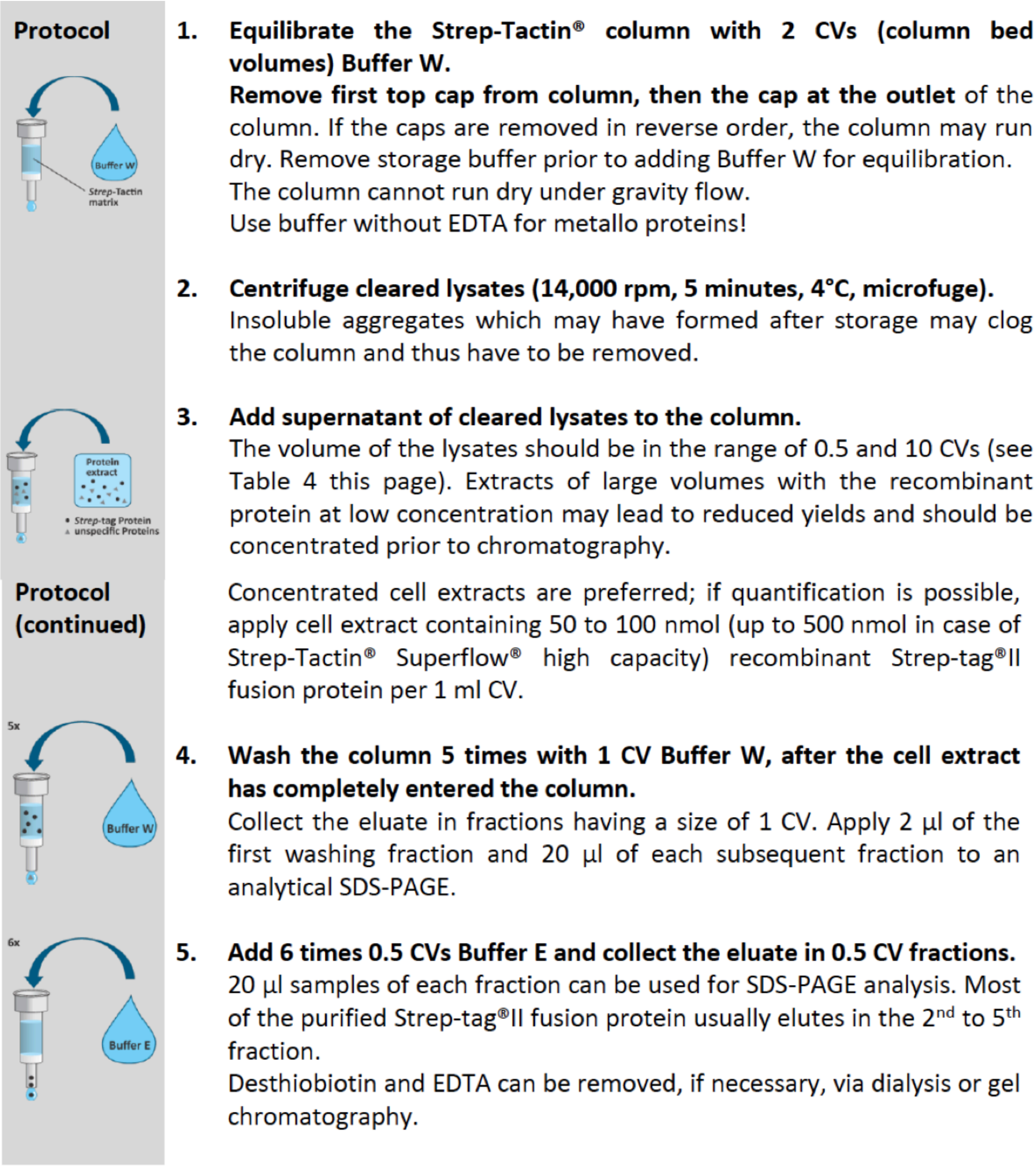
Note

For storage, antimicrobial agents should be added to the column to prevent contamination ([M] 0.001 % (v/v) phenyl mercuric salts, [M] 0.005 % (v/v) thimerosal, [M] 0.05 % (v/v) chlorobutanol, [M] 0.002 % (v/v) chlorhexine, [M] 0.02 % (v/v) sodium azide, or [M] 20 % (v/v) ethanol are acceptable).
When necessary, the gel can be removed from the column and sterilized by autoclaving.

9

PURIFICATION & ELUTION

31. Purification and elution of dPGM uses a Strep-tactin Sepharose, 20 mL bed column, as detailed in the manufacturer's instructions:



Protocol

1. **Equilibrate the Strep-Tactin® column with 2 CVs (column bed volumes) Buffer W.**
   Remove first top cap from column, then the cap at the outlet of the column. If the caps are removed in reverse order, the column may run dry. Remove storage buffer prior to adding Buffer W for equilibration.
   The column cannot run dry under gravity flow.
   Use buffer without EDTA for metallo proteins!

2. **Centrifuge cleared lysates (14,000 rpm, 5 minutes, 4°C, microfuge).**
   Insoluble aggregates which may have formed after storage may clog the column and thus have to be removed.

3. **Add supernatant of cleared lysates to the column.**
   The volume of the lysates should be in the range of 0.5 and 10 CVs (see Table 4 this page). Extracts of large volumes with the recombinant protein at low concentration may lead to reduced yields and should be concentrated prior to chromatography.

Protocol (continued)

Concentrated cell extracts are preferred; if quantification is possible, apply cell extract containing 50 to 100 nmol (up to 500 nmol in case of Strep-Tactin® Superflow® high capacity) recombinant Strep-tag®II fusion protein per 1 ml CV.

4. **Wash the column 5 times with 1 CV Buffer W, after the cell extract has completely entered the column.**
   Collect the eluate in fractions having a size of 1 CV. Apply 2 µl of the first washing fraction and 20 µl of each subsequent fraction to an analytical SDS-PAGE.

5. **Add 6 times 0.5 CVs Buffer E and collect the eluate in 0.5 CV fractions.**
   20 µl samples of each fraction can be used for SDS-PAGE analysis. Most of the purified Strep-tag®II fusion protein usually elutes in the 2nd to 5th fraction.
   Desthiobiotin and EDTA can be removed, if necessary, via dialysis or gel chromatography.

Note

For purification and regeneration of Strep-tactin resin, see Appendices 3 and 4.

10 PRECIPITATION

32. Measure the volume of supernatant and add saturated ammonium sulfate to [M] 80 % (v/v)

33. Add the saturated ammonium sulfate drop-wise to the supernatant while stirring continuously using a separatory funnel in a ring stand. Stir for 30 min.

34. Collect precipitated material by centrifugation at 20000 x g, 4°C, 00:13:00 (slow brake).

35. Ressuspend pellet with 3 mL **buffer F.**

11 **DESALTING**

36. After equilibrating Sephadex G-25 fine column with 3 x column volume **buffer F**, load the sample and elute with **buffer F**, taking aliquots and checking the amount of protein with Bradford reagent (monitor the development of blue colour by eye for a qualitative assessment, not necessary to read absorbance).

Note

It is not necessary to add proteases inhibitors as now it is a pure protein.

37. Pool samples with highest protein content.

Note

Start adding 5 mL of **buffer F** and collecting 2 mL aliquots. Check TSP “by eye”.
In our hands, samples pooled were from fractions 3 to 6.

38. Once desalting of the sample is complete, wash column with 2 column volumes of [M] 0.2 Molarity (M) NaOH, rinse with water, and re-equilibrate with 2-3 column volumes of **buffer A**.

Note

For storage, antimicrobial agents should be added to the column to prevent contamination ([M] 0.001 % (v/v) phenyl mercuric salts, [M] 0.005 % (v/v) thimerosal, [M] 0.05 % (v/v) chlorobutanol, [M] 0.002 % (v/v) chlorhexine, [M] 0.02 % (v/v) sodium azide, or [M] 20 % (v/v) ethanol are acceptable).
When necessary, the gel can be removed from the column and sterilized by autoclaving.

39. Store pooled fractions at 🌡 -80 °C .

Note

Before freezing samples check the activity (3-PGA comsumption in presence of increasing concentrations of 2,3dPGA using the PK-LDH protocol in this series). It is important to keep an aliquot of previous dPGM preparation to compare results obtained.

APPENDIX

12 **APPENDIX 1. Purification of plasmid DNA, aka minipreps for sequencing Following Day 3**

1. Prepare a glycerol stock of the culture (for long term storage): combine 300 µL culture + 300 µL 30% glycerol in a 2 mL screw-cap tube, mix gently by inversion. Store at -80°C.
2. Spin the remaining cell culture down for 10 min at 4000 rpm, room temperature.
3. Pour off the supernatant into the bacterial waste bottle and leave tubes upside down and open on blue roll for a few minutes to drain any excess supernatant.
4. Resuspend the pellets in 250 µl Resuspension Solution (stored at 4°C) and transfer to labelled 1.5 ml tubes.
5. Vortex briefly to eliminate all clumps of bacteria.
6. Add 250 µl Lysis Solution, invert gently 4-6 times to mix, then incubate at RT for 4 min.
7. Add 350 µl Neutralisation Solution, invert gently 4-6 times to mix.
8. Spin in the microcentrifuge at 13,400 rpm for 5 min. Transfer the supernatant to a labelled spin column.
9. Spin in the microcentrifuge at 13,400 rpm for 1 min. Discard the flow-through.
10. Wash the column by adding 500 µl Wash Solution.
11. Spin in the microcentrifuge at 13,400 rpm for 1 min. Discard the flow-through.
12. Wash the column for a second time by adding another 500 µl Wash Solution.
13. Spin in the microcentrifuge at 13,400 rpm for 1 min. Discard the flow-through.
14. Spin the empty column in the microcentrifuge at 13,400 rpm for 1 min.
15. Transfer the column to a clean labelled 1.5 ml tube, add 55 µl SDW to the middle of the column and incubate at RT for 2 min. Spin in the microcentrifuge at 13,400 rpm for 1 min. Discard the column.
16. Determine the concentration and purity of pDNA using a nanodrop (DNA concentration, 260:230 nm ratio)

APPENDIX 2. SDS-Page for checking protein in different fractions

UNINDUCED Sample (from Induction step 10.)

1. Thaw pellet. Resuspend in about 100 µL of Lysis Buffer.
2. Vortex and incubate on ice.
3. Rupture the cells and DNA by sonicating the suspension until solution becomes easy to pipette. Insert sonicator half way into the solution and apply 3 × 5 s bursts, with the tube always on ice. Pause for some seconds between each burst.
4. Centrifuge at 4000 rpm for 10 min at 4°C. Keep SN and pellet.
5. Quantify the total soluble portein (TSP) in the supernatant. Add Loading Buffer (proportion of 4:5 LB:Sample), incubate at 95°C for 4 min. Dilute to 0.5 mgTSP/mL with blank (Lysis buffer + Loading Buffer).
6. Resuspend pellet in 100 µL of Lysis Buffer. Add Loading Buffer (proportion of 4:5 LB:Sample), incubate at 95°C for 4 min.

INDUCED Sample (from Lysis step #21.)

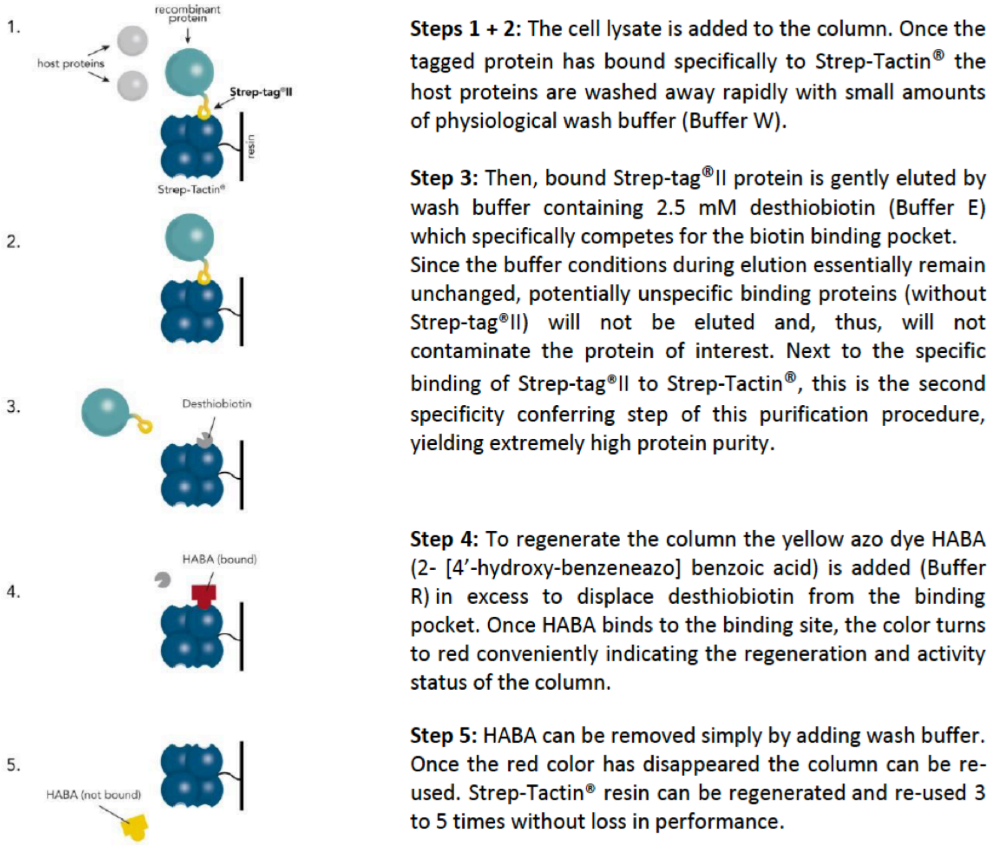
1. After sonication, centrifuge at 4000 rpm for 10 min at 4°C. Keep supernatant and pellet.
2. Quantify TSP in the supernatant. Add Loading Buffer (proportion of 4:5 LB:Sample), incubate at 95°C for 4 min. Dilute to 0.5 mgTSP/mL with blank (Lysis buffer + Loading Buffer).
3. Resuspend pellet in 100 µL of Lysis Buffer. Add Loading Buffer (proportion of 4:5 LB:Sample), incubate at 95°C for 4 min.

Run samples on SDS-Page and stain with coomassie blue to visulaise proteins.

Note

In our hands, loading 2 mg TSP/lane provides clear protein bands. The protein is expected to be in the soluble fraction, but some protein can be seen in the insoluble fraction. Note that the gel is just qualitative for the insoluble protein fraction.

APPENDIX 3. Step-tactin purification process, as detailed in the manufacturer's instructions:



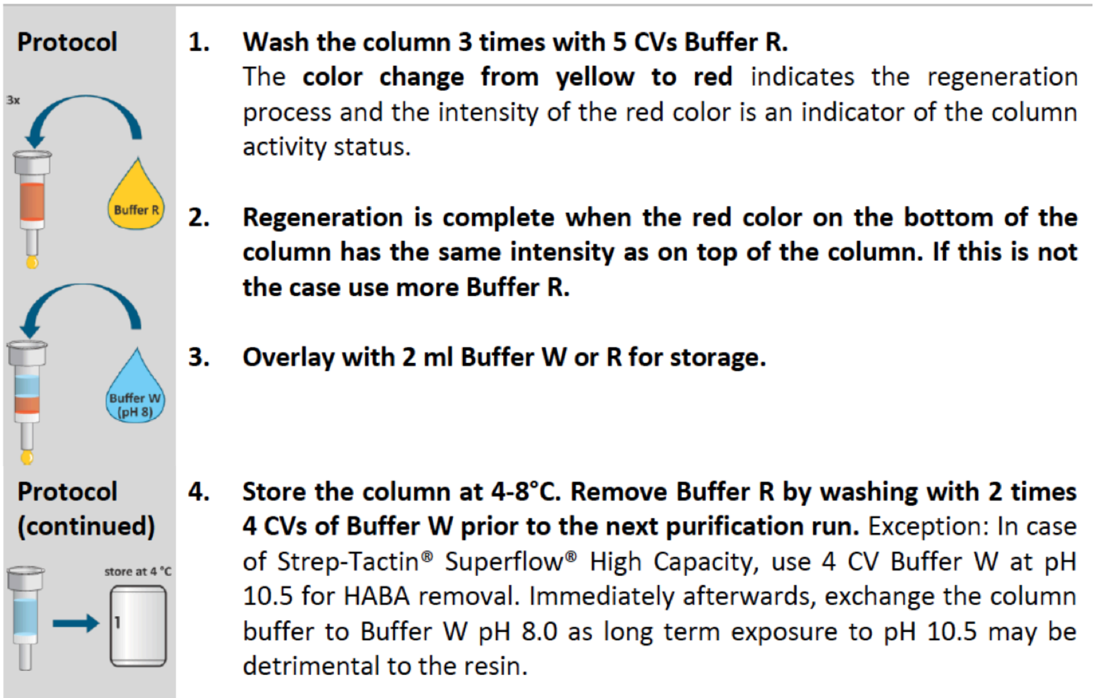
Steps 1 + 2: The cell lysate is added to the column. Once the tagged protein has bound specifically to Strep-Tactin® the host proteins are washed away rapidly with small amounts of physiological wash buffer (Buffer W).

Step 3: Then, bound Strep-tag®II protein is gently eluted by wash buffer containing 2.5 mM desthiobiotin (Buffer E) which specifically competes for the biotin binding pocket. Since the buffer conditions during elution essentially remain unchanged, potentially unspecific binding proteins (without Strep-tag®II) will not be eluted and, thus, will not contaminate the protein of interest. Next to the specific binding of Strep-tag®II to Strep-Tactin®, this is the second specificity conferring step of this purification procedure, yielding extremely high protein purity.

Step 4: To regenerate the column the yellow azo dye HABA (2- [4'-hydroxy-benzeneazo] benzoic acid) is added (Buffer R) in excess to displace desthiobiotin from the binding pocket. Once HABA binds to the binding site, the color turns to red conveniently indicating the regeneration and activity status of the column.

Step 5: HABA can be removed simply by adding wash buffer. Once the red color has disappeared the column can be re-used. Strep-Tactin® resin can be regenerated and re-used 3 to 5 times without loss in performance.

APPENDIX 4. Regeneration of Strep-tactin resin, as detailed in the manufacturer's instructions:



Protocol

1. **Wash the column 3 times with 5 CVs Buffer R.**
   The **color change from yellow to red** indicates the regeneration process and the intensity of the red color is an indicator of the column activity status.

2. **Regeneration is complete when the red color on the bottom of the column has the same intensity as on top of the column. If this is not the case use more Buffer R.**

3. **Overlay with 2 ml Buffer W or R for storage.**

Protocol (continued)

4. **Store the column at 4-8°C. Remove Buffer R by washing with 2 times 4 CVs of Buffer W prior to the next purification run.** Exception: In case of Strep-Tactin® Superflow® High Capacity, use 4 CV Buffer W at pH 10.5 for HABA removal. Immediately afterwards, exchange the column buffer to Buffer W pH 8.0 as long term exposure to pH 10.5 may be detrimental to the resin.

Citations

Scales JC, Parry MA, Salvucci ME. A non-radioactive method for measuring Rubisco activase activity in the presence of variable ATP: ADP ratios, including modifications for measuring the activity and activation state of Rubisco
https://doi.org/10.1007/s11120-013-9964-5

IBA Life Sciences. Expression and purification of proteins using Strep-Tactin. A comprehensive manual
https://www.iba-lifesciences.com/isotope/2/2-1002-100-Manual_Strep-Tactin-Purification.pdf

van de Loo FJ, Salvucci ME. Activation of ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) involves Rubisco activase Trp16
10.1021/bi9604901

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White MF, Fothergill-Gilmore LA. Development of a mutagenesis, expression and purification system for yeast phosphoglycerate mutase
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