Extended Protocol

RapidPro FPLC™ 5 mL

QCD-029

*All changes must be recorded. This is a master, living, document. Printed copies/PDFs are not controlled.

Kit Components

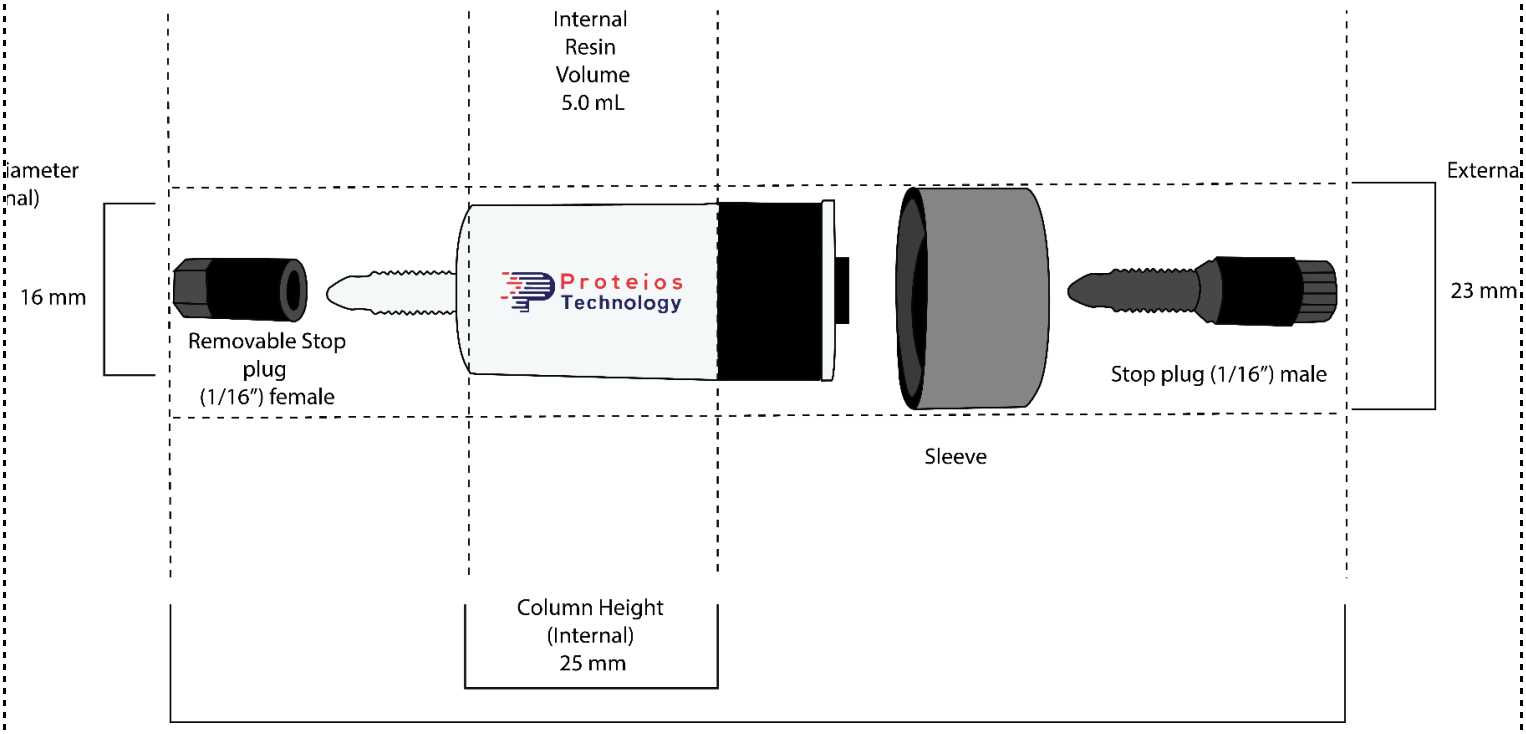
1. 250 mL **L1** Lysis Buffer (Qty 1)
2. 250 mL **W2** Wash Buffer (Qty 1)
3. 250 mL **E1** Elution Buffer (Qty 1)
4. 5 mL pre-packed FPLC Columns (Qty **2**)

Introduction

Proteios RapidPro FPLC™ purification kit is based on the interaction between the Proteios Car9 affinity tag and the Proteios RapidPro FPLC™ Resin.

The RapidPro FPLC™ Kit has been optimized to provide high-purity isolation of high to low expressed proteins in *E. coli* allowing the purification of Car9-tagged protein with an average purity > 90%.

Figure 1. Proteios RapidPro FPLC™ column volume (CV) 5 mL. Column dimensions 1.6 × 2.5 cm. Columns are delivered with a stop plug at the inlet and a snap off end at the outlet. The columns must be stored at 4°C for optimal performance and shelf life.



Column Performance Characteristics

Matrix	Proteios RapidPro FPLC™ resins
Particle Size	250-500 µm
Binding Capacity	40 mg/mL
Recommended Flow Rate	5 mL/min
Column Pressure	0.5 MPa
Column Stability	Stable in all commonly used aqueous buffers, reducing agents, and detergents.
pH working range	5.0-8.5
Storage	4°C - 8°C
Expiration	Up to 6 months

Cloning

The target protein of interest (POI) can be expressed with a Proteios Car9 tag at either the N-terminal or C-terminal position. Proteios Car9-tagged POI binds to the Proteios FPLC Resin and can be released with the addition of the Proteios Elution Buffer.

Proteios Car9 DNA sequence (GATAGCGCACGCGGTTTCAAAAAACCGGGTAAACGC) coding for DSARGFKKPGKR and the Proteios' spacer (AAGCTTGGCGGCGGCTCT) coding for GGGS can be introduced at the N-terminal of the protein of interest after the start codon ATG.

Alternatively, they can be introduced to the C-terminal before any stop codon(s) - first the spacer (AAGCTTGGCGGCGGCTCT) and then the Proteios Car9 coding sequence (GATAGCGCACGCGGTTTCAAAAAACCGGGTAAACGC).

In addition to the spacer reported above, other spacers might be introduced between the CDS (Coding Sequence) of the protein of interest and the Proteios Car9 CDS.

We recommend gene synthesis and subcloning to insert both Proteios Car9 and Spacer CDS in frame with the protein of interest CDS. Alternatively, a PCR-based approach can be used to insert both Proteios Car9 and spacer CDS in the desired position prior to the subcloning of the new sequence in the desired expression plasmid.

We strongly recommend to sequence verify the insert sequence after cloning into your preferred expression plasmid.

The sequence verified expression plasmid may be transformed into Top10 chemically competent cells (Thermo Fisher) or analog cell line to amplify plasmid DNA according to manufacturing instructions. Commercially available plasmid purification kits can be used to extract and purify the plasmid DNA.

Cell Culture

BL21(DE3) or similar cell lines can be used to express the POI according to manufacturing instructions. Proteios has successfully tested them with both IPTG and arabinose induction systems. Usually, 1 mM IPTG or 0.1% arabinose are enough to induce protein expression.

1. Transform BL21(DE3) or similar competent cell lines according to the manufacturer's instruction.

2. Plate the transformed bacteria on agar Petri plates with appropriate antibiotics to obtain about 100 colonies on the plate.

3. Grow colonies overnight at 37°C.

4. The next day, pick a colony and grow it overnight in 5 mL of LB media at 37°C in a shaking incubator at 200 rpm. Addition of appropriate antibiotics is recommended.

5. The next morning, add 1 mL of overnight culture to 50 mL of media and incubate at 37°C in a shaking incubator at 200 rpm. Addition of appropriate antibiotics is recommended.

6. Grow bacteria until they reach the desired OD600 value.

7. Add IPTG or Arabinose to induce protein expression (other induction systems can be used).

8. Grow cells for the required time and temperature to allow the expression of the POI.

9. Harvest the cells by centrifugation, remove supernatant and either proceed to the next step or freeze cell pellets and store at -20°C.

Cell Lysis

Proteios Purification Buffer does not contain: EDTA, DNase, reducing agents and protease inhibitors. Proteios Lysis buffer is compatible with up to 500 mM of NaCl addition.

You can supplement the Proteios' Buffers with the desired amounts of these supplements as needed.

Proteios Protein Purification Buffer has been tested with high-pressure homogenization, sonication and freeze/thaw.

Resuspend the cells in Proteios Protein Purification buffer; 4 mL of buffer is recommended for a pellet obtained from a 50 mL bacteria culture (about 10 mL of lysis buffer /g of bacteria pellet).

To lyse bacteria, **use 1** of these 4 recommended methods:

1. Microfluidizer homogenizer
2. High-pressure homogenizer: 3 passages on French Press, 540 psi
3. Sonication: 6 cycles of sonication on ice bath by alternating 5 seconds on and 30 seconds off at 30% power
4. Freeze/thaw: 3 cycles of freeze and thaw by alternating -80°C and 37°C. Note: Freeze at -80°C until solid, then thaw at 37°C until liquid, vortex before next freezing step.

Depending on the target protein, one method may be more suitable than the others.

Usually freeze/thaw leads to a lower yield and higher purity.

Centrifuge the cell lysate at 10000 x g for 15 minutes at 4°C.

The supernatant can be directly loaded on the equilibrated FPLC column.

Protein Purification

Proteios FPLC Columns contain Proteios silica-based resin.

To prevent air from entering the column bed, **remove the top stop plug first** and ***then*** *twist off the bottom end*.

The columns should be equilibrated prior to adding the clarified lysate.

It is recommended to equilibrate the columns immediately especially when the protein purification is performed at room temperature, because air bubbles can be formed due to exposure to a higher temperature from a lower temperature.

Please see the purification steps below.

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QCD-028 Intended use-For research use only. Revision Date: 2023 July 27

Before Starting

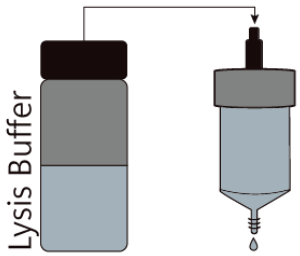
- Cloning
- Cell Culture
- Cell lysis. Thaw lysate.

See Extended Protocol for Details.

Kit Components

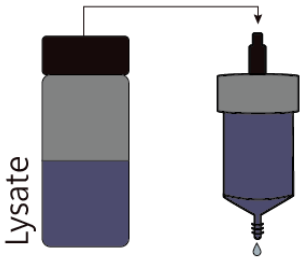
- 250 mL L1 Lysis Buffer (Qty 1)
- 250 mL W2 Wash Buffer (Qty 1)
- 250 mL E1 Elution Buffer (Qty 1)
- 5 mL pre-packed FPLC Columns (Qty 2)

Purification steps



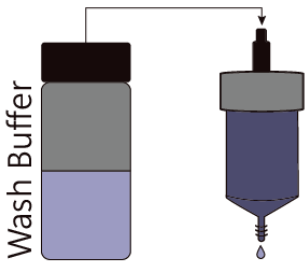
1. Equilibration: Equilibrate the column.

- Connect the column to an FPLC system or a syringe pump and equilibrate with 5 column volumes (CVs) of Proteios RapidPro FPLC™ Lysis buffer (approximately 25 mL for a 5 mL column).
- Recommended equilibration flow rate is 5 mL/min.
- Discard the Flow Through (FT).



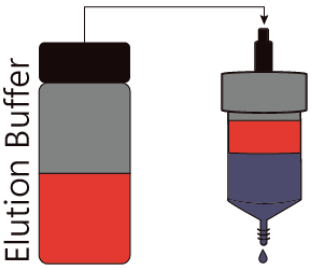
2. Binding: Load Lysate on the column.

- (Recommended: save from 20 µL to 40 µL of lysate for SDS gel.)
- Load Lysate on the column. (If required, filter the lysate through a 0.2 µm syringe filter since the large lysate volumes contaminated with debris may lead to impure yield.)
- Recommended lysate volume range is 0.5 CVs to 10 CVs.The binding flow rate is 0.5 mL/min.
- Discard any remaining FT.



3. Washing: Wash the column.

- Wash the column with Proteios RapidPro FPLC™ Wash buffer until the FPLC system's measuring absorbances reach a stable plateau.
- **In the case of a syringe pump**, ten times the lysate volume is recommended as the washing volume.
- The washing flow rate is 5 mL/min. Discard the FT.



4. Elution: Elute the Protein of Interest.

- Use approximately 6 CVs of Proteios RapidPro FPLC™ Elution buffer. Recommended flow rate for Elution is 1.0 mL/min.
- Collect all the eluate in 0.5-2.0 mL fraction volumes. The Protein of interest (POI) is in the eluate.
- Analyze all the fractions in SDS-PAGE.
- After use, wash the column with 10 CVs of distilled water to store. SEE EXTENDED PROTOCOL FOR REGENERATION PROCEDURE.

Column Regeneration

Proteios FPLC columns can be regenerated and reused *up to* **5 *times*** without losing performance.

Regeneration Buffer- 1 M NaCl in autoclaved DI water.

Column regeneration is required before each run.

- Regenerate the column by washing it with 3 CVs of DI water followed by 3 CVs of 1.0 M NaCl solution. Next, wash with 6 CV distilled water.
- Use a flow rate of 2.5 mL/min with NaCl and 5 mL/min for DI water.

Modifications/Troubleshooting

Problem	Suggestion
POI does not bind the matrix	•Add up to 500 mM NaCl to the Lysis Buffer. •Add up to 10% glycerol and/or up to 0.3% Tween 20 to the Lysis Buffer. •Extend the length of the spacer between Proteios Car9 and POI.
POI purity is < 90%	•Lyse cells using freeze/thaw. •Perform washes using 1:10 or 1:5 dilution of 1X Elution Buffer in the 1X Protein Purification Buffer.