Extended Protocol

RapidPro MiniSpin™

Protein Purification Kit

QCD-048

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

Kit Components

1. 125 mL **L1** Lysis Buffer (Qty 1)
2. 125 mL **W2** Wash Buffer (Qty 1)
3. 125 mL **E1** Elution Buffer (Qty 1)
4. Pre-packed MiniSpin Columns (Qty 10)

Introduction

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

The Proteios RapidPro MiniSpin™ Purification Kit has been optimized to provide high purity isolation of high- to very high-expressed proteins in *E. coli*. Purification takes approximately 30 minutes with an average purity > 98%.

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 RapidPro MiniSpin™ 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 Lysis Buffer does not contain: EDTA, DNase, reducing agents and protease inhibitors.

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

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

Resuspend the cells in Proteios Lysis 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 for about 5 minutes, 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 10,000 x g for 15 minutes at 4°C.

The supernatant can be directly loaded on the equilibrated RapidPro MiniSpin™ column.

Quick Protocol RapidPro MiniSpin™

QCD-047 Intended use-For research use only. Revision Date: 2023 July 27

Before Starting

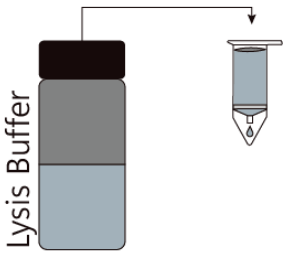
- Cloning
- Cell Culture
- Cell lysis. Thaw lysate.

See Extended Protocol for Details.

Kit Components

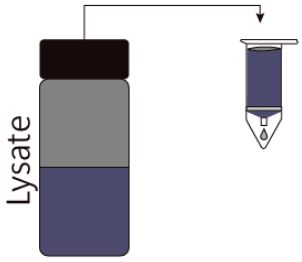
- 125 mL L1 Lysis Buffer (Qty 1)
- 125 mL W2 Wash Buffer (Qty 1)
- 125 mL E1 Elution Buffer (Qty 1)
- Pre-packed MiniSpin Columns (Qty 10)

Purification steps



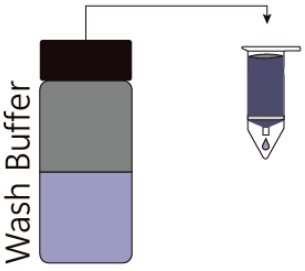
1. Equilibration: Equilibrate the column.

- Proteios RapidPro MiniSpin™ Columns are shipped dry and should be equilibrated prior to adding the clarified lysate.
- Add 600 µL of Proteios RapidPro MiniSpin™ Lysis Buffer to the column, centrifuge at 4000 x g for 3 minutes at room temperature.
- Discard the flow through (FT).



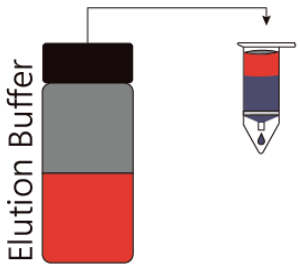
2. Binding: Load Lysate on the column.

- (Recommended: save from 20 µL to 40 µL of lysate for SDS gel.)
- Load 600 µL of lysate to the column.
- Centrifuge the column at 4000 x g for 3 minutes at room temperature.
- Discard the FT.



3. Washing: Wash the column.

- Wash the column with 600 µL of RapidPro MiniSpin™ Wash Buffer two times.
- Centrifuge for 3 minutes at 6000 x g at room temperature for each wash.
- Discard the FT.



4. Elution: Elute the Protein of Interest.

- Replace the collection tube with an empty microcentrifuge tube with cap for elution step.
- Add 300 µL RapidPro MiniSpin™ Elution Buffer to the column, centrifuge at 4000 x g for 3 minutes at room temperature.
- The Protein of interest (POI) is in the eluate.
- Repeat Elution step one more time to recover the remaining POI.)

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. • A mixture of Wash Buffer and Elution Buffer (such as 10:1) can be used to find the optimal purity/yield balance.