Protocol for Proteios LEP Purification Kit

Introduction

Proteios' Low-Expressed Protein (LEP) purification kit is based on the interaction between the Proteios Car9 affinity tag and the Proteios LEP Resin. The Proteios LEP Purification Kit has been optimized to provide high-purity isolation of very low- to moderate-expressed proteins in *E. coli*. You are able to purify up to 2mg of protein in about 15 minutes with an average purity > 90%.

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 LEP Resin and can be released with the addition of the Proteios LEP Elution Buffer.

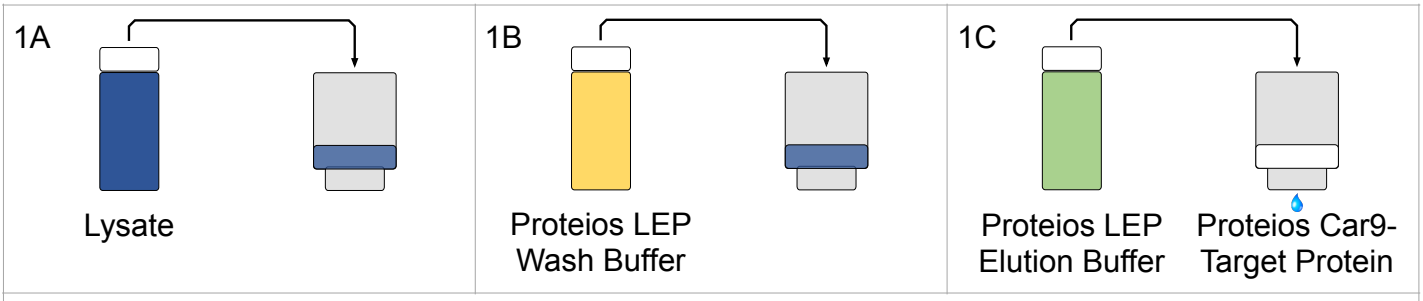

Figure 1: Technology overview. **1A** Protein lysate is loaded into the column and spun at 500g for 2 minutes. The POI is captured on the Proteios LEP Resin. **1B** Proteios LEP Wash Buffer is added to remove contaminants. **1C** The POI is eluted using Proteios LEP Elution Buffer.

Cloning

Proteios Car9 DNA sequence (`GATAGCGCACGCGGTTTCAAAAAACCGGGTAAACGC`) coding for `DSARGFKKPGKR` and the Proteios' spacer (`AAGCTTGGCGGCGGCTCT`) coding for `KLGGGS` 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 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. Verify the insert sequence after cloning into your preferred expression plasmid. 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. 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 mini prep 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, 1mM IPTG or 0.1% arabinose are enough to induce protein expression. 3h-4h induction at 37ºC and O/N induction at room temperature have also been successfully tested.

1. Transform BL21(DE3) or similar cell lines according to the manufacturer's instruction.
2. Plate the transformed bacteria on agar Petri plates with appropriate antibiotic to obtain about 100 colonies on the plate.
3. Grow colonies overnight at 37ºC.
4. The next day, pick a colony and grow it overnight in 5mL of LB media at 37ºC in a shaking incubator at 200rpm. Addition of appropriate antibiotic is recommended.
5. The next morning add 2.5mL of overnight culture to 50mL of media and incubate at 37ºC in a shaking incubator at 200rpm. Addition of appropriate antibiotic is recommended.
6. Grow bacteria until the OD600 reaches 0.6 (usually after 2 to 3 hours).
7. Add 0.1mM IPTG or 0.1% Arabinose.
8. Grow cells for 3 to 4 hours.
9. Centrifuge the bacteria culture at 3500g for 15min, remove supernatant and freeze cell pellet.

Cell Lysis

Proteios LEP Lysis Buffer does not contain either EDTA or proteinase inhibitors. If these are required, you can supplement the Proteios LEP Lysis Buffer with EDTA at the final concentration of 2mM and PMSF at the final concentration of 1mM just prior to use. Store the remaining Proteios LEP Lysis Buffer without EDTA and PMSF. Other proteinase inhibitor cocktails may be used as well. We suggest using 4mL Proteios LEP Lysis Buffer for a 50mL culture pellet.

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

1. Suspend the frozen pellet in 4mL of Proteios LEP Lysis Buffer. 4mL is recommended for a pellet obtained from a 50mL bacteria culture.
2. To lyse bacteria, use:
   - *High-pressure homogenizer*: 3 passages on French Press, 540 psi.

     OR
   - *Sonication*: 6 cycles of sonication on ice bath by alternating 5 seconds on and 30 seconds off at 30% power.

     OR
   - *Freeze/thaw*: 3 cycles of freeze and thaw by alternating -80ºC and 37ºC.

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.

3. Centrifuge the cell lysate at 10000g for 15 minutes at 4ºC. The supernatant can be directly loaded on the equilibrated purification column.

Protein Purification

The Proteios LEP Purification Kit provides up to 2mg of very low- to moderate-expressed protein in about 15 minutes with an average purity > 90%. It requires a centrifuge able to spin a 50mL tube at 500g.

Proteios LEP Columns containing Proteios LEP resin are shipped dry and should be equilibrated prior to adding the clarified lysate.

1. *Equilibrate the column*. Add 4mL of Proteios LEP Lysis Buffer to the column, centrifuge at 500g for 2 minutes at room temperature. Discard the flow through.
2. *Load Lysate on the column*. Load 4mL of lysate to the column and spin the column at 500g for 2 minutes at room temperature.
3. *Washing*. Wash the column with 4mL of Proteios LEP Wash Buffer three times. Spin for 2 minutes at 500g at room temperature for each wash. Discard the flow through.
4. *Elution*. Add 4mL Proteios LEP Elution Buffer to the column, centrifuge at 500g for 2 minutes at room temperature. The Proteios Car9-tagged POI is in the eluate. Repeat Elution step one more time to recover the remaining Proteios Car9-tagged POI.
5. *Dialysis and concentration*. Proceed to dialyze and/or concentrate the purified protein of interest.

The Proteios LEP Elution Buffer contains L-Lysine; we recommend to dialyze the purified protein for optimal protein concentration determination. Alternatively, filter spin columns can be used to concentrate and exchange buffers. Usually, the majority of the POI is in the first elution.

SDS-PAGE is recommended to evaluate the success of the purification.

Modifications/Troubleshooting

Problem	Suggestion
POI does not bind the matrix	• Add up to 500mM 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 binds the matrix but it is eluted during washes	• Lysis Buffer can be used to perform the washes instead of Wash Buffer. • A mixture of Lysis Buffer and Wash Buffer can be used to find the optimal purity/yield balance.
POI purity is < 80%	• Lyse cells using freeze/thaw.