CL7/Im7 1-mL Gravity Flow Column Purification

- Short Protocol -

TRIALTUS
BIOSCIENCE

Version 1.2
Last modified 11/23/20

trialtusbioscience.com

CL7/Im7 1-mL Gravity Flow Column Purification

The CL7/Im7 purification method is based on the ultra-high-affinity interaction of Colicin E7 DNAse and its inhibitor Immunity Protein 7 (Im7). CL7, a variant of CE7 that has no DNA binding or DNAse activity, binds to Im7 with a K_d of ~10^{-14} – 10^{-17} M. The ultra-high affinity interaction of the CL7/Im7 method enables high recovery and purity (>97%) in a single chromatography step.

This system outperforms His-trap and other traditional methods in several areas. CL7/ Im7 is stable in high salt loading buffers which are effective at removing impurities. CL7 and Im7 are also highly specific to only each other. This prevents nonspecific interactions between the tag or ligand and cellular components such as DNA and other proteins. The combination of these two factors, high salt tolerance and highly specific binding, results in such high purity that the CL7/Im7 system requires only one chromatography step.

Buffer Composition:

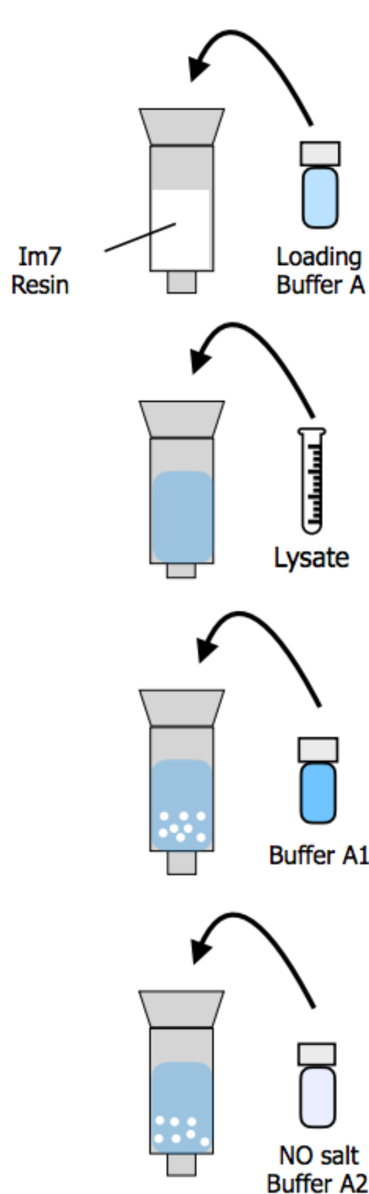
Loading Buffer A (Equilibration, Lysis, Elution Buffer): 0.5 M NaCl, 20 mM Tris, pH 8.0, 5% glycerol*
Washing Buffer A1 (High salt): 2 M NaCl, 20 mM Tris, pH 8.0, 5% glycerol
Washing Buffer A2 (No salt): 0 M NaCl, 20 mM Tris, pH 8.0, 5% glycerol
Elution Buffer A3: 0.2-0.5 M NaCl, 20 mM Tris, pH 8.0, 5% glycerol, 0.2 mM EDTA*
Gentle Elution Buffer: 3.6 M $MgCl_2$, 20 mM MES, pH 6.6

*Download the CL7/Im7 Expression & Purification Protocol for a more detailed protocol, exact concentrations, and troubleshooting based on type of protein.
https://trialtusbioscience.com/pages/protein-purification-protocols

Licensing Information
TriAltus Bioscience holds the exclusive, worldwide license to the CL7 protein purification technology platform. It was licensed from the University of Alabama at Birmingham (UAB) in Birmingham, Alabama, USA. An international patent filing has been made with protection being sought in the United States, Europe, and other major markets. The CL7 purification technology is available for research use. For commercial use or resale, contact us at info@trialtusbioscience.com to discuss commercial licensing.
Trademarks
The Company name, the terms "Trialtus" and "Trialtus Bioscience", the Company logo, and all related names, logos, product and service names, designs, and slogans are trademarks of the Company or its affiliates or licensors. You must not use such marks without the prior written permission of the Company. All other names, logos, product and service names, designs, and slogans on this Website are the trademarks of their respective owners.

CL7/Im7 Purification for 1-mL GFC Kits

(Short Protocol | Refer to website for more detailed protocols)



1. **Equilibrate the column:** Equilibrate column with 5 mL Loading Buffer A*

   Note: Loading Buffer A with 0.5M NaCl is not always the optimal buffer - it largely depends on the target protein properties. Please refer to one of the comprehensive protocols for more details.

2. **Load lysate on the Im7 column:** Add 80 mL of lysate (from 4 g cells) to Im7 column. Wash the column with two, 10 mL cycles of Loading Buffer A before proceeding with no salt/high salt washes.

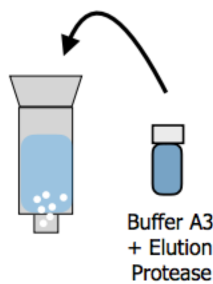
3. **High salt washing (Buffer A1):** Wash Im7 column with 12 mL of Washing Buffer A1 (high salt).

4. **NO salt washing (Buffer A2):** Wash the Im7 column with 12 mL of Washing Buffer A2 (no salt).

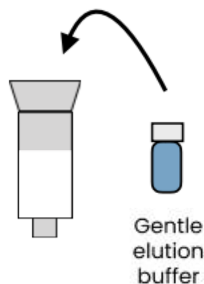
5. **Washing cycles:** Repeat step 3 and step 4, 2-3 times.

CL7/Im7 Purification for 1-mL GFC Kits

(Short Protocol | Refer to website for more detailed protocols)



6 **Proteolytic elution (Buffer A3):** Wash the column with two 10 mL cycles of Buffer A3. Leave 0.5-1.0 mL of Buffer A3 on top of the beads before adding the elution protease. Add the elution protease mixed with Buffer A3 and perform proteolytic elution for ~1.5-2.5 hrs (see Elution Protocol in Section 4 of one of the more detailed protocols for more information).



7 **Regeneration (Gentle Elution Buffer):** Wash the column with 12 mL of Gentle Elution Buffer to remove the CL7 tag. Regeneration is complete when no more protein elutes from the column (by OD280); wash with an extra 4-5 mL after the OD280 is stable to ensure no extra tag is left. Equilibrate column in 35 mL Loading Buffer A.