myTXTL® Protocol

His-tag Purification of Proteins from myTXTL® Reactions

Beads: HisPur™ Ni-NTA Magnetic Beads (Cat. No 88831, ThermoFisher)

Binding Buffer Composition:
- PBS (Phosphate buffered saline)

Wash Buffer 1 Composition:
- 100 mM Na2HPO4-NaH2PO4 (pH 8.0) (or potassium phosphate equivalent)
- 450 mM NaCl

Wash Buffer 2 Composition:
- 100 mM Na2HPO4-NaH2PO4 (pH 8.0) (or potassium phosphate equivalent)
- 600 mM NaCl
- 10-20 mM Imidazole (pH 8.0)

Elution Buffer Composition:
- 100 mM K2HPO4-KH2PO4 (pH 8.0) (or sodium phosphate equivalent)
- 600 mM NaCl
- 350 mM Imidazole (pH 8.0)

His-Bead Purification Protocol	
Sample Preparation	1. Following myTXTL expression, centrifuge myTXTL reactions at 16,000 xg for 3 minutes to pellet any insoluble material. Then, place reactions on ice. a. If insoluble material is a significant concern, transfer the supernatant into a new tube 2. Dilute 10-20 µL of myTXTL reaction product to 100 µL with Binding Buffer and place at room temperature (RT).
Bead Equilibration	1. For Pro Master Mix samples, transfer 40 µL of the stock Bead slurry into a 1.5-mL tube and for Antibody/DS Master Mix samples, transfer 20 µL. a. Ensure the stock Bead slurry is mixed well before transfer. 2. Add 160 µL Binding Buffer to the Bead slurry and vortex for 10 seconds at mid power. 3. Pulldown the beads with a magnetic stand and discard the supernatant. 4. Add 200 µL Binding Buffer to the Beads and vortex the beads for 10 seconds at mid power. 5. Pulldown the beads with a magnetic stand and discard the supernatant.
Sample Binding	1. Add 100 µL Sample to the beads and vortex for 5 seconds at medium-low power. 2. Shake the sample with beads for 10 minutes in a ThermoMixer at 1400 RPM, RT. a. Alternately, tap the sample periodically to suspend beads. 3. Pulldown the beads with a magnetic stand and discard the supernatant.
Washing	1. Add 400 µL Wash Buffer 1 to the Bead sample and vortex for 10 seconds at mid power. 2. Pulldown the beads with a magnetic stand and discard the supernatant. 3. Add 400 µL Wash Buffer 2 to the Bead sample and vortex for 10 seconds at mid power. 4. Pulldown the beads with a magnetic stand and discard the supernatant. 5. Add 200 µL Wash Buffer 2 to the Bead sample and vortex for 10 seconds at medium-low power. 6. Pulldown the beads with a magnetic stand and discard the supernatant.

His-Bead Purification Protocol *cont.*

Elution	1. Add 25 µL Elution Buffer to the Bead sample and vortex briefly at mid power. 2. Shake the sample with beads for 5 minutes in a ThermoMixer at 1450 RPM, RT. a. Alternately, tap the sample periodically to suspend beads. 3. Pulldown the beads with a magnetic stand and transfer the eluate to a new 1.5-mL tube. a. This eluted volume should contain your His-tagged protein. b. Load 2-9 µL of the eluate in an SDS-PAGE gel to visualize your target protein.

Appendix: Endotoxin Removal

Endotoxin levels can be severely depleted, to about 10-20 EU/mL, using a simple wash procedure during HisPur Magbead Purification

Materials	**Detergent**: TritonX-114 **Triton Wash Buffer** Composition: - 100 mM Na2HPO4-NaH2PO4 (pH 8.0) (or potassium phosphate) - 450 mM NaCl - 1.25% Triton (%v/v) Store at 4°C
Endotoxin Removal Washing	1. During HisPur purification, replace the first wash buffer with 400 µL Triton Wash Buffer and incubate the sample at 4-10°C for 10 minutes using a ThermoMixer at 1400 RPM. 2. Pulldown the beads with a magnetic stand and discard the supernatant. 3. Perform the standard HisPur washing steps for Wash 2 and 3.

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Questions? Contact us via the methods listed below. Our team is happy to assist you!

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