

# myTXTL<sup>®</sup> Protocol

## Protein A Purification of Proteins from myTXTL<sup>®</sup> Reactions

**Beads:** Protein A MagBeads (Cat. No L00273, GenScript)

**Binding/Wash Buffer:**

- Gentle Binding Buffer (ThermoFisher, Cat. No 21020)

**Elution Buffer:**

- Gentle Elution Buffer (ThermoFisher, Cat. No 21027)

Paired Kit of Gentle Binding and Elution Buffers: ThermoFisher, Cat. No 21030

### Protein A Bead Purification Protocol

<b>Sample Preparation</b>	<ol style="list-style-type: none"><li>1. Following myTXTL expression, centrifuge myTXTL reactions at 16,000 xg for 3 minutes to pellet any insoluble material. Then, place reactions on ice.<ol style="list-style-type: none"><li>a. If insoluble material is a significant concern, transfer the supernatant into a new tube</li></ol></li><li>2. Dilute 10-30 <math>\mu</math>L of myTXTL reaction product 4x with Binding Buffer and place at room temperature (RT).</li></ol>
<b>Bead Equilibration</b>	<ol style="list-style-type: none"><li>1. Transfer 5 <math>\mu</math>L of the stock Bead slurry into a 1.5-mL tube.<ol style="list-style-type: none"><li>a. Ensure the stock Bead slurry is mixed well before transfer.</li></ol></li><li>2. Pulldown the beads with a magnetic stand and discard the supernatant.</li><li>3. Add 50 <math>\mu</math>L Binding Buffer to the Bead slurry and vortex for 5 seconds at mid power.</li><li>4. Pulldown the beads with a magnetic stand and discard the supernatant.</li><li>5. Repeat Bead Equilibration Steps 3 and 4 twice more to equilibrate beads.</li></ol>
<b>Sample Binding</b>	<ol style="list-style-type: none"><li>1. Add the diluted myTXTL Sample to the beads and vortex for 5 seconds at medium-low power.</li><li>2. Shake the sample with beads for 30 minutes in a ThermoMixer at 1400 RPM, RT.<ol style="list-style-type: none"><li>a. Alternately, tap the sample periodically to suspend beads.</li></ol></li><li>3. Pulldown the beads with a magnetic stand and discard the supernatant.</li></ol>
<b>Washing</b>	<ol style="list-style-type: none"><li>1. Add 100 <math>\mu</math>L Wash Buffer to the Bead sample and vortex for 10 seconds at medium-low power.</li><li>2. Pulldown the beads with a magnetic stand and discard the supernatant.</li><li>3. Repeat Washing Steps 1 and 2 twice more to wash the bead sample.</li></ol>
<b>Elution</b>	<ol style="list-style-type: none"><li>1. Add 25 <math>\mu</math>L Elution Buffer to the Bead sample and vortex briefly at mid power.</li><li>2. Shake the sample with beads for 5 minutes in a ThermoMixer at 1450 RPM, RT.<ol style="list-style-type: none"><li>a. Alternately, tap the sample periodically to suspend beads.</li></ol></li><li>3. Pulldown the beads with a magnetic stand and transfer the eluate to a new 1.5-mL tube.</li><li>4. Elution Steps 1-3 may be repeated for a second elution to recover additional target protein.</li></ol>

## Protein A Bead Purification Protocol *cont.*

<p><b>Elution</b></p>	<p>Note: Gentle Elution Buffer contains high salt content and will form precipitate if mixed with phosphate buffers or SDS-PAGE sample loading dye. To prepare sample for SDS-PAGE, first perform buffer exchange.</p> <p>This protocol covers use of a 0.5 mL 30K MWCO spin filter. Ensure the MWCO used is appropriate for the target protein.</p> <ol style="list-style-type: none"> <li>1. Equilibrate a 0.5 mL spin filter with 500 uL TBS or HBS.             <ol style="list-style-type: none"> <li>a. Centrifuge for 3 minutes at 10,000 xg and discard the flowthrough and concentrate.</li> </ol> </li> <li>2. Add 225 uL TBS or HBS to the spin filter and dilute the 25 uL eluate into the TBS or HBS.             <ol style="list-style-type: none"> <li>a. Centrifuge for 3.5 minutes at 10,000 xg and remove the flowthrough.</li> </ol> </li> <li>3. Add 450 uL TBS or HBS to the spin filter, seal and invert 10x to mix.             <ol style="list-style-type: none"> <li>a. Centrifuge for 3.5 minutes at 10,000 xg and remove the flowthrough.</li> </ol> </li> <li>4. Repeat step 3 once more for a total buffer exchange to TBS or HBS of 1000-fold.</li> </ol>
-----------------------	---

## Appendix: Endotoxin Removal

<p>Endotoxin levels can be severely depleted, to about 10-20 EU/mL, using a simple wash procedure during Protein A Purification</p>	
<p><b>Materials</b></p>	<p><b>Detergent:</b> TritonX-114  <b>Triton Wash Buffer</b> Composition: 1.25% Triton (%v/v) dissolved in Gentle Binding Buffer and stored at 4°C.</p>
<p><b>Endotoxin Removal Washing</b></p>	<ol style="list-style-type: none"> <li>1. During Protein A purification, replace the first wash buffer with 100 µL Triton Wash Buffer and incubate the sample at 4-10°C for 10 minutes using a ThermoMixer at 1400 RPM.</li> <li>2. Pulldown the beads with a magnetic stand and discard the supernatant.</li> <li>3. Perform the standard Protein A washing steps with Gentle Binding Buffer for Wash 2 and 3.</li> </ol>

For research use only. Daicel and Daicel Arbor Biosciences are registered trademarks of Daicel Corporation.

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



**Web:** [www.arborbiosci.com](http://www.arborbiosci.com)  
**Email:** [info@arbor.daicel.com](mailto:info@arbor.daicel.com)  
**Phone:** 1-734-998-0751  
**X:** @ArborBio  
**Bluesky:** @arborbio.bsky.social



Rev. Date 02/2026