

# myTXTL<sup>®</sup> Protocol

## Strep-tag<sup>®</sup> Purification of Proteins from myTXTL<sup>®</sup> Reactions

**Beads:** MagStrep<sup>®</sup> Strep-Tactin<sup>®</sup>XT beads (Cat. No. 2-5090-002, IBA Lifesciences)

**Binding/Wash Buffer** Composition:

- 100 mM Tris-HCl (pH 8.0)
- 150 mM NaCl
- 1 mM EDTA

**Elution Buffer Concentrate:** Buffer BXT, 10x (Cat. No. 2-1042-025, IBA Lifesciences)

**Elution Buffer BXT (1x)** Composition:

- 100 mM Tris-HCl (pH 8.0)
- 150 mM NaCl
- 1 mM EDTA
- 50 mM Biotin

*Note: Strep-tag<sup>®</sup> based purification from myTXTL will result in co-purification of a 12 kDa protein with no known enzymatic activity.*

Strep-Tactin <sup>®</sup> XT 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 10-20 <math>\mu</math>L of myTXTL reaction product to 70 <math>\mu</math>L with Binding Buffer and place at room temperature (RT).</li></ol>
<b>Bead Equilibration</b>	<ol style="list-style-type: none"><li>1. Transfer 20 <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 100 <math>\mu</math>L Binding Buffer to the Beads 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 70 <math>\mu</math>L diluted myTXTL Sample to the beads and vortex for 5 seconds at medium-low power.</li><li>2. Shake the sample with beads for 15 minutes in a ThermoMixer at 1500 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>

## Strep-Tactin®XT Bead Purification Protocol *cont.*

<b>Elution</b>	<ol style="list-style-type: none"><li>1. Add 25 µL 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 1600 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></ol>
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**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



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