Protein Purification Protocol

1. Prepare buffers A and B.

Components	Buffer A (at least 100 mL)		Buffer B (at least 10 mL)	
	Concentration		Concentration	
HEPES	50	mM	50	mM
Ammonium chloride	1000	mM	-	-
Magnesium chloride	10	mM	10	mM
Potassium chloride	-	-	100	mM
Imidazole (pH=7)	-	-	500	mM
TCEP*	1	mM	1	mM

* Add just before use

2. Equilibrate the charged column with 30 mL of buffer A. After 25 mL of buffer A have passed through, close the column from the bottom. In parallel, continue with steps 3-5.
3. Thaw the cells and use a serological pipette to resuspend the cell pellet in 7.5 mL of buffer A.
4. Lyse the cells using a 130-watt probe sonicator with the following parameters: 4 x 20 s pulse on, 20 s pulse off, 70% amplitude.

NOTE: Make sure to keep the cells on ice during sonication. Place the probe deep enough into the solution without touching the tube. If a large amount of foam is generated, the energy transfer will be damped. In that case, let the foam settle, lower the probe deeper into the solution, and extend the sonication time.

5. Remove the cell debris by centrifugation at 21130 x g (max speed) for 20 min at 4 °C immediately after sonication. Keep the lysate on ice.
6. Add the supernatant to the equilibrated column.
7. Open the column from the bottom and let unbound components elute from the column.
8. Wash with 25 mL of buffer A.
9. Wash the column with 25 mL of wash buffer (25 mM imidazole buffer: 23.75 mL of buffer A + 1.25 mL of buffer B).
10. Elute the proteins with 5 mL of elution buffer (450 mM imidazole buffer: 0.5 mL of buffer A + 4.5 mL of buffer B). Collect the eluate in a Falcon tube. Keep the eluted protein on ice at all times.