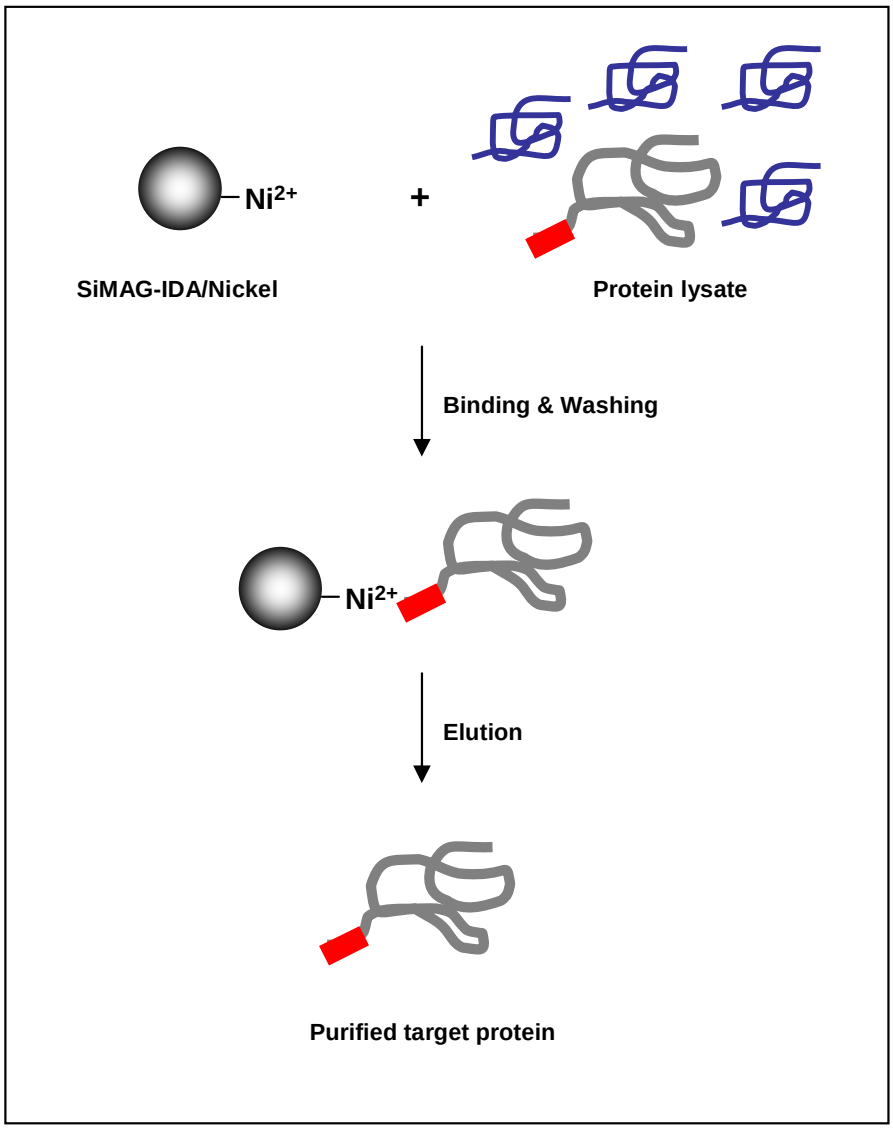
Purification of 6xHis-tagged proteins with magnetic SiMAG-IDA/Nickel particles

Introduction:

SiMAG-IDA/Nickel is designed for purification of 6xHis-tagged proteins.

The His-Tag sequence binds to the Ni^{2+} cations, which are immobilized on the **SiMAG-IDA/Nickel** particles. After unbound proteins are washed away, the target protein is recovered by elution with imidazole.



Equipment & Reagents:

- **SiMAG-IDA/Nickel** (25 mg/ml in ddH_2O, 20% Ethanol)
- **Binding capacity:** ~ 30 µg His-tagged protein / mg SiMAG-IDA/Nickel
- **Wash & Binding buffer (W & B buffer):**
  500 mM NaCl, 100 mM HEPES, 20 mM imidazole, pH 8.0
- **Elution buffer:** 100 mM HEPES, 500 mM imidazole, pH 8.0
- **Strip buffer:** 100 mM EDTA, 500 mM NaCl, 20 mM Tris-HCl, pH 7.9
- **Recharging buffer:** 100 mM $NiSO_4$
- **Magnetic separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Protocol:

The following general protocol describes the purification of recombinant 6xHis-tagged proteins under native conditions with SiMAG-IDA/Nickel

1. Wash 100 µl **SiMAG-IDA/Nickel** particles three times with 0.5 ml **W & B buffer** by magnetic separation and resuspend the beads in 0.5 ml **W & B buffer** by vortexing.
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2. To this suspension add the 6xHis-tagged protein solution (max. final volume of 1.0 ml) with a required amount of the 6xHis-tagged protein based on the binding capacity of the particles.
3. Incubate at room temperature for 15-30 minutes by gentle mixing.
4. Place the tube in the magnetic separator and discard the supernatant.
5. Add 1 ml **W & B buffer** and gentle mix the suspension by pipetting up and down, collect the beads for 30 seconds with the magnetic separator, remove and discard the supernatant and repeat the washing step two times.
6. After the last wash step, resuspend the 6xHis-tagged protein coated beads in 100 µl **Elution buffer**.
7. Incubate at room temperature for 5 minutes by gentle mixing.

8. Place the tube in the magnetic separator and transfer the supernatant in a clean tube.

Note: Multiple elution steps will maximize your yield of target protein.

9. For regeneration of the particles wash three times with 1.0 ml **Strip buffer**.

10. Place the tube in the magnetic separator and remove and discard the supernatant.

Add 1 ml ddH_2O, mix the suspension by vortexing, collect the beads for 30 seconds with the magnetic separator, remove and discard the supernatant.

Add 1 ml **Recharging buffer** to recharge the particle matrix, mix the suspension by vortexing, collect the beads for 30 seconds with the magnetic separator, remove and discard the supernatant, repeat this step.

Add 1 ml ddH_2O, mix the suspension by vortexing, collect the beads for 30 seconds with the magnetic separator, remove and discard the supernatant, repeat this step.

Final regeneration step: Resuspend the particles in ddH_2O, 20% Ethanol.

11. After the last wash, resuspend the reused particles in 100 µl ddH_2O, 20% Ethanol.

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