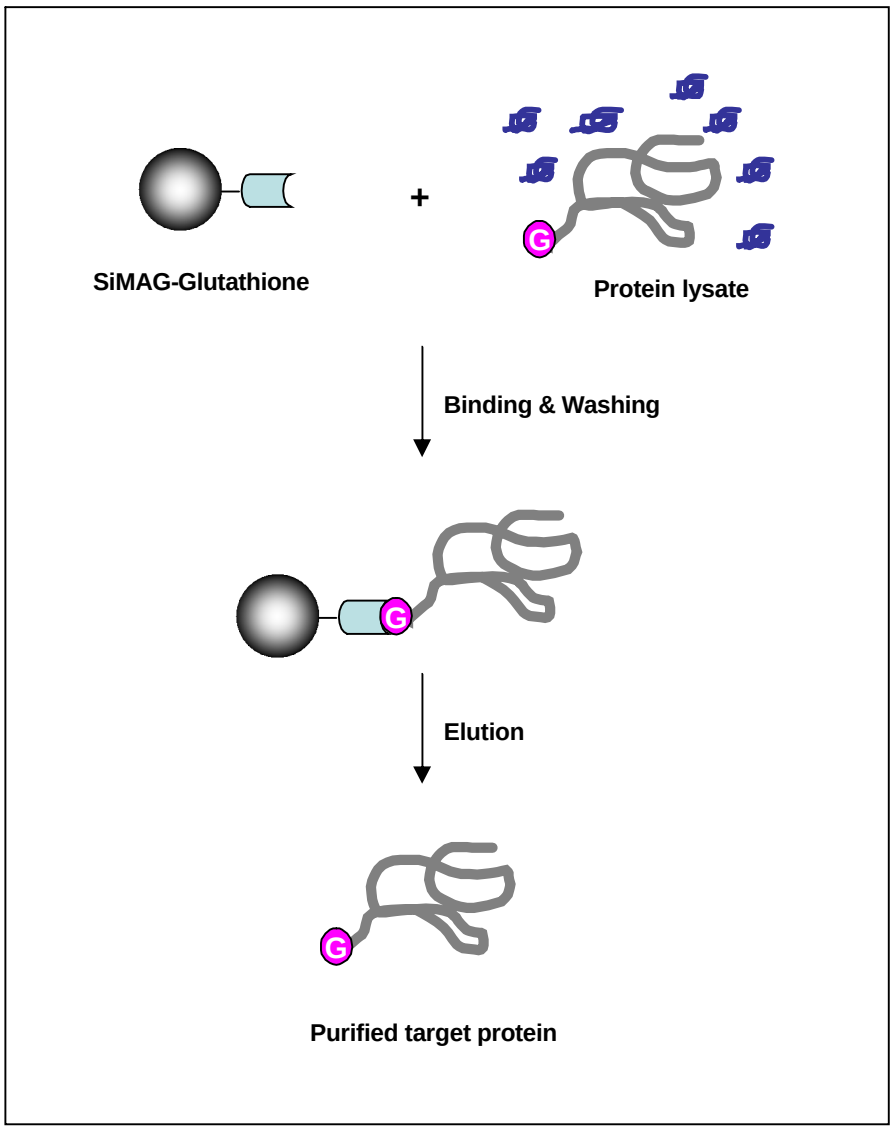
Purification of GST-fusion proteins with magnetic SiMAG-Glutathione particles

Introduction:

SiMAG-Glutathione is designed for rapid purification of recombinant glutathione S-transferase (GST) fusion proteins from bacteria lysates and other S-transferases or glutathione-dependent proteins.

The GST-fusion protein which is bound to **SiMAG-Glutathione** particles can be easily collected by means of a **MagnetoPURE** separators, which enables binding, wash and elution procedures to be carried out in a single tube.



Equipment & Reagents:

- **SiMAG-Glutathione** (25 mg/ml in ddH_2O, 0.05 % sodium azide)
- **Binding capacity:** ~ 100 µg GST-Tag fusion protein / 1 mg SiMAG-Glutathione
- **Wash & Binding buffer (W & B buffer):**
  Phosphate Buffered Saline (PBS), pH 8.0
- **Elution buffer:** 50 mM Tris-HCl, pH 8.0, 25 mM Glutathione (reduced)

  Note: Prepare the **Elution buffer** immediately before use. Depending on the molecular weight of the bound GST-fusion protein, the concentration of Glutathione can be increased from 10 mM for small proteins up to 100 mM for large proteins.
- **Regeneration buffer I:** 50 mM Tris-HCl, 0.5 M NaCl, pH 8.0
- **Regeneration buffer II:** 100 mM sodium acetate, 0.5 M NaCl, pH 4.5
- **Magnetic separator** (**e.g. MagnetoPURE,** Product Number: MP-10)

Protocol:

The following general protocol describes the purification of recombinant glutathione S-transferase (GST) fusion proteins under native conditions with SiMAG-Glutathione

1. Wash 100 µl **SiMAG-Glutathione** particles three times with 1.0 ml **W & B buffer** by magnetic separation and resuspend the beads in 0.25 ml **W & B buffer** by vortexing.

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2. Transfer the particle suspension to the GST-fusion protein containing solution (max. final volume 1.5 ml).
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 vortexing or pipetting (up and down), collect the particles for 30 seconds with the magnetic separator, remove and discard the supernatant and repeat the washing step two times.

6. After the last washing step, resuspend the particles in 100 µl **Elution buffer**.

7. Incubate at room temperature for 15 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 with 1.0 ml **Regeneration buffer I** by use of the magnetic separator as described above.

10. Wash the magnetic particles with 1.0 ml **Regeneration buffer II.**

11. After the last wash, resuspend the reusable particles in 100 µl ddH_2O, 0.05 % sodium azide.

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