

Detection of protein-protein interactions by GST pulldown with PureCube Glutathione MagBeads

Overview

This protocol describes the pulldown of proteins interacting with a GST-tagged bait protein with Glutathione MagBeads. Magnetic beads are well-suited for pull-down experiments, because they are easy to separate from the medium, which speeds up the entire process.

In this protocol, the GST-tagged bait protein is expressed in *E.coli* cells, and immobilized on Glutathione magnetic beads. The prey protein is typically obtained from mammalian cell lysates or cell-free reactions, and captured by interaction with the bait protein on the magnetic beads. Elution can be done either by incubation with reduced glutathione, or, if SDS-PAGE is the only detection method, with SDS-PAGE buffer.

Note that the buffer conditions described in this protocol may need to be optimized, depending on the proteins analyzed. Binding of the bait protein, interaction between bait and prey protein, and elution conditions may be highly variable. Also the amount of cell lysates needed for preparing bait and prey protein may vary depending on expression levels. To monitor binding efficiencies of bait and prey proteins, samples should be analyzed from various steps in the workflow.

To minimize false positive results, it is important to use negative controls, e.g. magnetic beads without any bait protein, or, even better, GST protein expressed in *E.coli* without bait protein fused.

Detection of the interacting proteins can be done e.g. by SDS-PAGE and Western Blot if specific antibodies for the expected prey protein are available. Alternatively, the prey protein can be synthesized in a cell-free reaction in the presence of radioactive (e.g. ³⁵S-labeled) amino acids for subsequent detection.

Please contact us if you have questions or need assistance optimizing a protocol for your application (contact@cube-biotech.com); other protocols can also be found at www.cube-biotech.com/protocols.

Equipment

- Ice bath
- Refrigerated centrifuge (min 10,000xg)
- Micropipettor
- Micropipetting tips
- 1.5 mL conical microcentrifuge tubes
- Magnetic holder for microcentrifuge tubes (for separation of magnetic beads)
- pH meter
- Vortex
- End-over-end shaker
- SDS-PAGE equipment
- Optional: Western Blot equipment

Materials

- Cell pellet from *E.coli* cells expressing the GST-fused bait protein (e.g., from 1-2 mL culture)
- Cell pellet from *E.coli* cells expressing GST as negative control (e.g. from 1-2 mL culture)
- PureCube Glutathione MagBeads (Cube #32201)
- Sodium phosphate monobasic (NaH₂PO₄)
- Sodium chloride (NaCl)
- Sodium hydroxide (NaOH)
- Lysozyme
- Benzonase[®] nuclease (e.g. Merck Milipore #707464)
- Protease inhibitor cocktail (e.g., Roche cOmplete, #04693116001)
- Dithiothreitol (DTT)
- Glycerol
- Sodium dodecyl sulfate (SDS)
- Bromophenol blue
- Tris base
- Hydrochloric acid (HCl)
- EDTA (e.g. Cube Biotech #61262)
- Triton X-100
- Optional: Reduced Glutathione (e.g. Cube Biotech # 61033)
- Optional: Igepal CA-630 (Nonidet P40)
- Optional: Tween-20
- Optional: GST Antibody (Cube Biotech #40060)

Solutions and buffers

Lysis Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH ₂ PO ₄	50 mM	119.98	0.5 M	29.99 g/ 500 mL	10 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	3 mL
DTT	1 mM	154.25	1 M	1.54 g/ 10 mL	100 µL
EDTA	1 mM	292.24	0.5 M	14.6 g/100 mL	200 µL
Lysozyme	1 mg/mL		100 mg/mL	1 g / 10 mL	1 mL
Triton X-100	1% (v/v)		100%(v/v)		1 mL
Protease inhibitor	1x				2 tablets

Instructions: Mix in 60 mL water. Adjust the pH to 7.2 using NaOH and then add water to a total volume of 100 mL. Always prepare fresh.

Binding and Wash (BW) Buffer, 100 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
NaH ₂ PO ₄	50 mM	119.98	0.5 M	29.99 g/ 500 mL	10 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	3 mL

Instructions: Mix in 60 mL water. Adjust the pH to 7.2 using NaOH and then add water to a total volume of 100 mL.
Note: Optimal buffer conditions may vary depending on the protein of interest. Proteins may require addition of protease inhibitor cocktail, EDTA, 1-5 mM DTT, 1% BSA, or detergents such as 0.5-1% Igepal CA-630 (Nonidet P-40).

SDS-PAGE Elution Buffer, 1 mL

Instructions: Mix 200 µl 5XSDS-PAGE buffer with 800 µL BW Buffer.

Native Elution Buffer, 10 mL (optional)

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris base	125 mM	121.14	0.5 M	30.29/ 500 mL	2.5 mL
NaCl	150 mM	58.44	5 M	146.1 g/ 500 mL	300 µL
Triton X-100	0.1% (v/v)		100% (v/v)		10 µL
Reduced glutathione	50 mM	307.32			154 mg
DTT	1 mM	154.25	1 M	1.54 g/10 mL	10 µL

Instructions: Dissolve in 8 mL water, stir until the reduced glutathione is completely dissolved. Depending on your protein requirements, adjust pH to 7.5-8.0 with NaOH and add water to 10 mL. Always prepare fresh.

5X SDS-PAGE Buffer, 10 mL

Component	Final concentration	Molecular weight (g/mol)	Stock concentration	Amount needed for stock	Stock needed for buffer
Tris-HCl, pH 6.8–7.0	300 mM	121.14	1 M	12.11 g/ 100 mL	3 mL
Glycerol	50% (v/v)	–	100% (v/v)	–	5 mL
SDS	5% (w/v)	–	–	–	0.5 g
Bromophenol blue	0.05% (w/v)	–	4%	–	125 µL
DTT	250 mM	154.25	1 M	1.54 g/ 10 mL	125 µL/aliquot

Instructions: Prepare a 1 M Tris-HCl stock by dissolving Tris base in 60 mL deionized water, adding HCl to a pH of 6.8–7.0, and adding water to a final volume of 100 mL. For the SDS-PAGE Buffer, mix all components listed **except DTT** and add water to a total of 10 mL. Freeze aliquots (375 µL each) at –20 °C. Before use, add DTT to the needed single aliquots.

Procedure

1. Thaw the *E. coli* cell pellets containing the GST protein and the GST-fusion bait protein from 1–2 mL bacterial culture on ice. Perform all subsequent steps in parallel with the GST-fusion bait protein and the GST protein as negative control.
2. Resuspend the two cell pellets in 0.2 mL Lysis Buffer each. Add 3–6 U Benzonase® (3 units/mL bacterial culture) to the lysate to reduce viscosity caused by genomic DNA.
3. Incubate for 30 min on ice, if necessary for protein stability. Otherwise, incubating at room temperature (20–25 °C) may be more efficient.
4. Centrifuge the lysate for 30 min at 10,000xg and 2–8 °C. Collect the supernatant.
6. Resuspend the PureCube Glutathione MagBeads by vortexing. Transfer 20 µL each of the 25% magnetic beads suspension into two conical microcentrifuge tubes.
7. Add 250 µL of BW buffer and mix by vortexing. Place the tube on a magnetic microtube stand until the beads are separated and discard the supernatant.
8. Repeat step 7. This ensures that residual ethanol in the magnetic bead storage buffer is removed that might interfere with protein binding.
9. Resuspend the equilibrated MagBeads in 100 µL Buffer BW.
10. Pipet 200 µL of the cleared lysate onto the equilibrated magnetic beads, and incubate the lysate-magnetic bead mixture at 4 °C for 1 h on an end-over-end shaker.
11. Place the tube on the magnetic microtube stand. Allow the beads to separate and remove the supernatant.

Optional: Freezing the cell pellet at –20 °C for 30 min prior to incubation at room temperature improves lysis by lysozyme.

Note: The supernatant contains the soluble proteins and is the **cleared lysate fraction**. Check by SDS-PAGE analysis that the bait protein is in the cleared lysate fraction. If the bait protein is insoluble, expression conditions need to be optimized before proceeding with the experiment.

Note: Additives like 1–5 mM DTT, 1% BSA, 0.5–1% Igepal CA-630 or change in pH may support binding of the bait protein and reduce unspecific background. If your bait protein is prone to protease digest, add protease inhibitor cocktail and/or 1 mM EDTA to the sample. If your bait protein is a membrane protein, ensure that the required detergent is present in all steps.

12. Remove the tube from the magnet. Add 250 μ L Buffer BW and mix by vortexing. Place the tube again on the magnetic microtube stand and allow the beads to separate. Remove the supernatant.
13. Repeat step 12 twice, then resuspend the MagBeads in 20 μ L Buffer BW.
14. Use 5-10 μ L of the MagBeads per pull-down assay reaction. Add the mammalian cell lysate or cell-free reaction containing the bait protein in a volume of 20-50 μ L, and add Buffer BW to obtain a total volume of 200 μ L. (Example: 10 μ L MagBeads + 40 μ L cell lysate + 150 μ L Buffer BW = 200 μ L).
15. Incubate at 4°C for 1 h on an end-over-end shaker.
16. Briefly vortex the MagBeads, place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
17. Add 400 μ L of BW buffer and mix gently. Incubate at room temperature for 5 min, then place on the magnetic stand and allow the beads to separate. Remove the supernatant.
18. Repeat step 17 four more times.
19. Elute by adding 20 μ L of SDS-PAGE Elution Buffer. Vortex and incubate at room temperature for 5 min.
20. Place the tube on the magnetic microtube stand until the beads separate and remove the supernatant.
21. Analyze the supernatant by SDS-PAGE and Western Blot or radioactive detection.

For Western Blot, use 5-10 μ L of undiluted sample.

For radioactive detection, remove 2 μ L of the eluate, dilute by adding 18 μ L of SDS-PAGE Elution Buffer, and use 2-5 μ L of the diluted sample.

Always boil the samples for 5 min before SDS-PAGE.

Optional: Save the supernatant for SDS-PAGE analysis to check for successful binding of the bait protein.

Optional: Save a 3-5 μ L aliquot of the MagBeads, and analyze the sample by SDS-PAGE to confirm binding of the bait protein to the MagBeads.

Note: Dependent on the proteins analyzed, optimization of incubation temperature or buffer conditions (e.g. change of pH, addition of BSA, detergent, or DTT) may be necessary.

Optional: Analyse the supernatant to check for binding efficiency of the prey protein.

Optional: The 5 min incubation step may be omitted in the last four wash steps.

Optional: If the subsequent analysis requires folded protein, alternatively the elution can be performed by adding 20 μ L of elution buffer. Elution steps should be repeated 3 -5 times.

Note: If your bait or prey protein is a membrane protein, do not boil the sample, but incubate it at 46°C for 30 min.

