# GST-Trap_M for Immunoprecipitation of GST-Fusion Proteins

For the immunoprecipitation of GST-fusion-proteins from cellular extracts.
*Only for research applications, not for diagnostic or therapeutic use*

## 1. Introduction

For biochemical analyses including mass spectrometry and enzyme activity measurements GST-fusion proteins and their interacting factors can be isolated fast and efficiently in one step by immunoprecipitation using the GST-Trap. GST-Trap_M contains a small GST-binding protein covalently coupled to the surface of magnetic beads. It enables the purification of any protein of interest fused to GST.

## 2. Content

| Reagent | Code | Quantity |
|---|---|---|
| GST-Trap_M | stm-20 | 20 reactions (0.5 ml resin) |
| GST-Trap_M | stm-100 | 100 reactions (2.5 ml resin) |
| GST-Trap_M | stm-200 | 200 reactions (5 ml resin) |
| GST-Trap_M | stm-400 | 400 reactions (10 ml resin) |

## 3. Bead properties

Bead size: ~ 0.5 - 1 µm
Storage buffer: 1x PBS, 0.05% sodium azide
Binding capacity: 10 µl GST-Trap_M slurry binds 0.25 – 0.5 µg of GST

## 4. Stability and Storage

Shipped at ambient temperature. Upon receipt store at +4°C.
Stable for 6 month. Do not freeze.

## 5. Protocol

1. For one immunoprecipitation reaction resuspend cell pellet of 1 ml bacteria culture expressing GST in 100 - 200 µl lysis buffer by pipetting.

   *optional: add 1 mM PMSF and 0.1 mg/ml lysozyme to lysis buffer*

2. Rotate tube for 1h at 4°C.
3. Sonify cell pellet to disrupt bacterial membrane.
4. Spin cell lysate at 20.000x g for 5 -10 minutes at 4°C.
5. Transfer supernatant to a pre-cooled tube. Adjust volume with dilution buffer to 500 µl. Discard pellet.

   For immunoblot analysis dilute 30 µl cell lysate with 10 µl 4x SDS-sample buffer (→ refer to as input).

6. Equilibrate GST-Trap_M beads in dilution buffer. Resuspend magnetic particles by vortexing and transfer calculated volume (20 - 30 µl) in a new reaction cup with 250 µl ice cold dilution buffer. Magnetically separate until supernatant is clear and wash twice with 250 µl of cold dilution buffer.
7. Add cell lysate to equilibrated GST-Trap_M beads and incubate the GST-Trap_M beads with the cell lysate under constant mixing for 10 min – 2 h at room temperature or 4°C.

   *note: during incubation of protein sample with the GST-Trap_M the final concentration of detergents should not exceed 0.2% to avoid unspecific binding to the matrix*

8. Magnetically separate until supernatant is clear. For western blot analysis dilute 30 µl supernatant with 10 µl 2x SDS-sample buffer (→ refer to as non-bound). Discard remaining supernatant.

9. Wash beads two times with 500 µl ice cold wash buffer.

   *optional: increase salt concentration in the second washing step up to 500 mM*

10. Resuspend GST-Trap_M beads in 100 µl 2x SDS-Sample buffer or go to step 11.

11. Boil resuspended beads for 10 minutes at 95°C to dissociate the immunocomplexes from the beads. The beads can be collected by centrifugation at 2.500x g for 2 minutes at 4°C and SDS-PAGE is performed with the supernatant (→ refer to as bound).

12. *optional: elute bound proteins by adding 50 µl 0.2 M glycine pH 2.5 (incubation time: 30 sec under constant mixing) followed by centrifugation. Transfer the supernatant to a fresh cup and add 5 µl 1M Tris base (pH 10.4) for neutralization. To increase elution efficiency this step can be repeated.*

**Suggested buffer composition**

| Buffer | Composition |
|---|---|
| Lysis buffer | 10 mM Tris/Cl pH 7.5; 150 mM NaCl |
| Dilution buffer | 10 mM Tris/Cl pH 7.5; 150 mM NaCl |
| Wash buffer | 10 mM Tris/Cl pH 7.5; 150 mM NaCl |
| Elution buffer | 200 mM glycine pH 2.5 |

## Support/ Troubleshooting

Please refer to our FAQ section at *www.chromotek.com* or contact *support@chromotek.com*

## Related products

| GST Toolbox | code |
|---|---|
| GST-Trap_M Kit | stmk-20 |
| Blocked magnetic beads | bmp-20 |
| GST antibody | 6g9 |

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