

GrabIt[™] Kits

About the Kits

| | | |
|--|--------|---------|
| GrabIt [™] GST•Tag [™] Kit | 50 rxn | 71434-3 |
| GrabIt S•Tag [™] Kit | 50 rxn | 71435-3 |
| GrabIt T7•Tag [®] Kit | 50 rxn | 71433-3 |
| GrabIt Protein G Plus/Protein A Kit | 50 rxn | 71436-3 |

Description

The GrabIt Kits are designed for the study of protein:protein interactions using a pull-down method. Proteins that interact with GST•Tag, S•Tag, or T7•Tag fusion proteins are co-purified using tag-specific affinity agaroses, and retained on a spin filter. The complexes are eluted with sample buffer in preparation for SDS-polyacrylamide gel electrophoresis. An additional kit for antibody-based immunoprecipitation relies on Protein G Plus/Protein A agarose for capture. Table 1 and Table 2, below, list the binding capacities provided by the agaroses in the GrabIt Kits. CytoBuster[™] Protein Extraction Reagent is included for convenient preparation of cytoplasmic protein samples of mammalian and insect cells. The GrabIt Kits provide enough CytoBuster[™] Protein Extraction Reagent, specific agarose, wash buffer, spin filters with receiver tubes, and 4X SDS sample buffer for 50 assays.

Table 1. Binding capacities

| Agarose | mg/ml settled resin |
|--------------------------|---------------------|
| GST•Bind [™] | 5 |
| S-protein | 0.5 |
| T7•Tag | 0.3 |
| Protein G Plus/Protein A | 20 (IgG) |

Table 2. Protein G Plus/Protein A binding

| Strong | Weak | None |
|------------------------|-----------|-------------|
| Cat IgG | Dog | Chicken IgG |
| Bovine IgG | Human IgA | Human IgD |
| Goat | Human IgE | |
| Guinea Pig | Human IgM | |
| Horse | | |
| Human IgG ₁ | | |
| Human IgG ₂ | | |
| Human IgG ₃ | | |
| Human IgG ₄ | | |
| Mouse | | |
| Pig | | |
| Rabbit | | |
| Rat | | |
| Sheep | | |

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Components

GrabIt™ GST•Tag Kit

- 2.5 ml GST•Bind™ Resin (5 ml of a 50% v/v suspension in 20% ethanol)
- 50 ml CytoBuster™ Protein Extraction Reagent
- 100 ml GrabIt Wash Buffer (150 mM NaCl, 50 mM Tris-HCl, 0.1% NP-40, pH 7.5)
- 50 Spin Filters
- 100 Receiver Tubes
- 2 ml 4X SDS Sample Buffer (300 mM DTT, 250 mM Tris-HCl, 30% glycerol, 6% SDS, 0.02% bromophenol blue, pH 6.8)

GrabIt S•Tag Kit

- 2.5 ml S-protein Agarose (5 ml of a 50% v/v suspension in 150 mM NaCl, 50 mM Tris-HCl, 1 mM EDTA, 0.02% sodium azide, pH 7.5)
- 50 ml CytoBuster Protein Extraction Reagent
- 100 ml GrabIt Wash Buffer
- 50 Spin Filters
- 100 Receiver Tubes
- 2 ml 4X SDS Sample Buffer

GrabIt™ T7•Tag® Kit

- 1 ml T7•Tag Antibody Agarose (2 ml of a 50% v/v suspension in 137 mM NaCl, 4.29 mM Na₂HPO₄, 2.7 mM KCl, 1.47 mM KH₂PO₄, 0.1% Tween®-20, 0.02% sodium azide, pH 7.3)
- 50 ml CytoBuster Protein Extraction Reagent
- 100 ml GrabIt Wash Buffer
- 50 Spin Filters
- 100 Receiver Tubes
- 2 ml 4X SDS Sample Buffer

GrabIt Protein G Plus/Protein A Kit

- 2 × 0.5 ml Protein G Plus/Protein A Agarose Suspension (2 × 1.5 ml of 33% suspension in PBS containing 0.1% azide)
- 50 ml CytoBuster Protein Extraction Reagent
- 100 ml GrabIt Wash Buffer
- 50 Spin Filters
- 100 Receiver Tubes
- 2 ml 4X SDS Sample Buffer

Storage

Store GrabIt Wash Buffer and agarose at 4°C. Store 4X SDS Sample Buffer at -20°C. Store all other components at room temperature or 4°C.

Available separately

| | |
|--|-----------------|
| Protease Inhibitor Cocktail Set I | Cat. No. 539131 |
| Protease Inhibitor Cocktail Set III (without EDTA) | Cat. No. 539134 |

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GrabIt™ Protocol**Using CytoBuster™ Protein Extraction Reagent**

CytoBuster Protein Extraction Reagent is a proprietary formulation of detergents optimized for efficient extraction of soluble proteins from mammalian or insect cells. The unique composition of CytoBuster enables isolation of functionally active proteins without secondary treatment, such as sonication, or freeze-thaw.

CytoBuster can be used directly for protein extraction at room temperature. Protein extraction can be performed on ice and in the presence of protease inhibitors (Cat. Nos. 539131 or 539134).

Table 3. Determination of CytoBuster volume requirements

| Culture Format | Surface Area (cm ²) | Volume of CytoBuster |
|------------------|---------------------------------|----------------------|
| 96-well Plate | 0.3 | 30 µl |
| 48-well Plate | 0.8 | 50 µl |
| 24-well Plate | 2.0 | 100 µl |
| 12-well Plate | 4.0 | 200 µl |
| 6-well Plate | 9.6 | 300 µl |
| 35-mm Dish | 9.6 | 300 µl |
| 60-mm Dish | 21.0 | 500 µl |
| 100-mm Dish | 55.0 | 1.0 ml |
| T-25 Flask | 25.0 | 500 µl |
| T-75 Flask | 75.0 | 1.5 ml |
| Suspension cells | * | 150 µl |

* 10⁶ cells. Suspension cells vary greatly in cell size, thus, adjustment may be necessary.

Extraction of monolayer cells

- Aspirate culture medium from cells.
Optional: Wash cells once with PBS (137 mM NaCl, 10 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4) or Hanks Buffered Salts Solution (HBSS).
- Add recommended amount of CytoBuster (see Table 1).
- Incubate at room temperature for 5 min.
- To maximize recovery, scrape cell debris using cell scraper (rubber policeman). Orient plate so all debris is pooled in CytoBuster.
- Transfer extract to suitably sized tube and spin at 16,000 × g for 4 min at 4°C.
- Transfer supernatant (cell extract) to new tube and proceed with pull-down assay.

Note: Extracts prepared with CytoBuster can be used immediately, or frozen at –20°C or –80°C until needed. Store extracts at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.

Extraction of suspension cells

- Collect cells by low speed centrifugation (e.g., 5 min at 2,500 × g).
Optional: Wash cells once with PBS (137 mM NaCl, 10 mM Na₂HPO₄, 2.7 mM KCl, 1.8 mM KH₂PO₄, pH 7.4) or Hanks Buffered Salts Solution (HBSS). Collect cells by low speed centrifugation, as above, and discard supernatant. Drain cell pellet well.
- Resuspend cells in CytoBuster using 150 µl per 10⁶ cells (optimal amount of CytoBuster may vary, based on cell size).
- Incubate at room temperature for 5 min.
- Transfer solution to suitably sized tube and spin at 16,000 × g for 5 min at 4°C.
- Transfer supernatant (cell extract) to new tube and proceed with pull-down assay.

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Note: *Extracts prepared with CytoBuster™ Protein Extraction Reagent can be used immediately, or frozen at –20°C or –80°C until needed. Store extracts at a temperature compatible with target protein activity; some target proteins may be inactivated by freeze-thaw cycles.*

Pull-down assay protocol

The following protocol is for use with GrabIt™ GST•Tag™, S•Tag™, and T7•Tag® Kits.

1. Place 1 ml extract prepared with CytoBuster Protein Extraction Reagent in a 1.5-ml microcentrifuge tube.
2. Thoroughly resuspend tag-specific agarose. If using GST•Bind™ Resin or S-protein Agarose, add 100 µl slurry to extract. If using T7•Tag Antibody Agarose, add 40 µl slurry to extract. Place tube on a rocker or spin wheel at low rpm at 4°C for 1 h.
3. Prepare 100 µl 1X SDS Sample Buffer per reaction by combining 25 µl 4X SDS Sample Buffer and 75 µl water. Heat to 80°C.
4. Place Spin Filter in 2-ml Receiver Tube. Transfer 500 µl of extract/resin mixture to the Spin Filter.
5. Spin Receiver Tube/Spin Filter at 14,000 × g for 30 s.

Note: *Do not let agarose dry during procedure. Drying can reduce target protein yield. After centrifugation, proceed immediately to the next step.*

6. Add remaining extract/agarose mixture to the Spin Filter and spin, as in Step 5.
7. Empty Receiver Tube. Wash agarose three times by adding 500 µl GrabIt Wash Buffer directly to agarose. Between washes, spin Receiver Tube/Spin Filter at 14,000 × g for 30 s. Empty Receiver Tube after each spin.
8. Transfer Spin Filter to new Receiver Tube.
9. Add 50 µl 1X SDS Sample Buffer to agarose. Cap Receiver Tube/Spin Filter. Place at 80°C for 5 min.
10. Spin Receiver Tube/Spin Filter at 14,000 × g for 30 s.
11. Sample can be immediately loaded on gel for SDS-PAGE analysis, or stored at –20°C.

Immunoprecipitation protocol

The following protocols are for use with the GrabIt Protein G/Plus Protein A Kit.

Preabsorption procedure

Preabsorption is often performed to minimize extra bands resulting from nonspecific binding. If preabsorption is performed, the kit provides enough resin for 25 immunoprecipitations.

1. Place 1 ml extract prepared with CytoBuster Protein Extraction Reagent in 1.5-ml microcentrifuge tube.
2. **Optional:** Add 2.5 µg normal IgG from the same species as immunoprecipitating antibody and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 30 min.
3. Add 50 µl Protein G Plus/Protein A Agarose slurry to extract and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 1 h.
4. Place Spin Filter in 2-ml Receiver Tube. Transfer 500 µl extract/resin mixture to Spin Filter.
5. Spin Receiver Tube/Spin Filter at 14,000 × g for 30 s.
6. Add remaining extract/agarose mixture to Spin Filter and spin, as in Step 5.

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Immunoprecipitation

1. Place 1 ml extract prepared with CytoBuster™ Protein Extraction Reagent, or 1 ml preabsorbed extract in a 1.5-ml microcentrifuge tube.
2. Add 1–5 µg antibody specific to the target protein, and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 30 min.
3. Add 50 µl Protein G Plus/Protein A Agarose slurry to extract and mix by inverting tube. Place tube on rocker or spin wheel at low rpm at 4°C for 1 h.
4. Prepare 100 µl 1X SDS Sample Buffer per reaction by combining 25 µl 4X SDS Sample Buffer and 75 µl water. Heat to 80°C.
5. Place Spin Filter in 2-ml Receiver Tube. Transfer 500 µl extract/resin mixture to Spin Filter.
6. Spin Receiver Tube/Spin Filter at 14,000 × g for 30 s.

Note: Do not let resin dry during procedure. Drying can reduce yield of target proteins. After centrifugation proceed immediately to the next step.

7. Add remaining extract/agarose mixture to Spin Filter and spin, as in Step 5.
8. Empty the Receiver Tube. Wash the resin three times by adding 500 µl GrabIt™ Wash Buffer directly to agarose. Between washes, spin Receiver Tube/Spin Filter at 14,000 × g for 30 s. Empty Receiver Tube after each spin.
9. Transfer Spin Filter to new Receiver Tube.
10. Add 50 µl 1X SDS Sample Buffer to the agarose. Cap Receiver Tube and place at 80°C for 5 min.
11. Spin Receiver Tube/Spin Filter at 14,000 × g for 30 s.
12. Sample can be immediately loaded on gel for SDS-PAGE analysis, or stored at –20°C.

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