

How to conduct a GFP–fusion protein immunoprecipitation of *Arabidopsis thaliana* plant samples with ChromoTek’s GFP–Trap

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1. Introduction

Green Fluorescent Protein (GFP) has been used for decades as an invaluable tool in plant research not only for monitoring trafficking and subcellular localization of proteins of interest but also for identifying binding partners to the protein of interest in co-immunoprecipitations (Co-IP). This Application Note provides a short protocol for the use of GFP-Trap® Agarose for IPs from *Arabidopsis thaliana* samples. It is based on more than 70 peer reviewed publications available at <https://www.chromotek.com/references/>. Please note that the protocol may also serve as inspiration when working with other plants e.g. *Nicotiana* (see online references).

2. Protocol

This Application Note describes how to prepare harvested plant material for a pull-down of GFP-tagged fusion proteins (and potential binding partners when doing a Co-IP) with ChromoTek's GFP-Trap Agarose. Prior to this, the target protein vector (containing the GFP-fusion protein sequence) has been transformed into plant cells using standard transformation methods. Then, the transformed plant cell culture has been cultivated and harvested.

2.1. Cell material

The amount of sample material depends on the expression level of the GFP-tagged fusion protein. For one IP reaction it is recommended to use 1-10 g of seedling or leaf material. Alternatively, 1-20 mg of total protein extracts can be used.

2.1. Materials and buffer composition

This protocol describes the immunoprecipitation with GFP-Trap® Agarose (product code: gta). GFP-Trap® Magnetic Agarose (product code: gtma) or GFP-Trap® Magnetic Particles M-270 (product code: gtd) can alternatively be used because they contain the same anti-GFP Nanobody as GFP-Trap Agarose. The handling of magnetic beads would be adapted from this protocol so that separation is accomplished by a magnet instead of centrifugation. See the corresponding manuals for additional information.

Suggested buffer composition:

Lysis buffer	<ul style="list-style-type: none"> ▶ 50 mM Tris/HCl pH 7.5, 150 mM NaCl OR 25 mM Hepes/KOH pH 7.4, 100 mM NaCl ▶ supplements (depending on protein of interest): <ul style="list-style-type: none"> ○ 10-15 mM MgCl₂ ○ 5-15% glycerol ○ 0.5-2 mM EDTA ○ 1-10 mM DTT ○ 1% Triton™ X-100 or 0.1- 0.5% Nonidet™ P-40 Substitute ○ 1 mM PMSF or 1x protease inhibitor cocktail, 1x phosphatase inhibitor cocktail
Wash buffer	same as the lysis buffer
2x SDS-sample buffer	120 mM Tris/HCl pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol

2.2. Cell lysis

1. Harvest plant material and grind into a fine powder in liquid nitrogen.
2. Transfer powder to a 15 mL tube.
Optional: Store in a freezer.
3. Resuspend powder in lysis buffer: 1-3 mL lysis buffer per 1 g powder.
4. Mix by vortexing for 1 min, incubate for 30 min at +4°C / on ice.
Optional: Pass lysate through a 1 mL syringe with needle to further facilitate cell lysis.
5. Remove cell debris by centrifugation (e.g. 20.000x g 15 min, +4°C).
Optional: Clear lysate additionally by filtration.
6. Submit cleared lysate to IP.

2.3. Immunoprecipitation with GFP-Trap

Bead equilibration

1. Resuspend GFP-Trap Agarose beads by gently pipetting up and down. Do not vortex the beads!
2. Transfer bead slurry into an appropriate reaction tube.
3. It is recommended to start with 25 μ L of bead slurry for one IP reaction.
If there is still GFP-tagged protein left in the flow-through fraction, increase the volume of bead slurry to 50 μ L per IP.
4. Add 500 μ L ice-cold lysis buffer.
5. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C and discard supernatant.

Protein binding

1. Add cleared lysate to the equilibrated beads.
If required, save 50 μ L of supernatant for further analysis (input fraction).
2. Rotate end-over-end for 1 hour at +4°C.

Washing

1. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C.
If required, save 50 μ L of supernatant for further analysis (flow-through/non-bound fraction).
2. Discard remaining supernatant.
3. Resuspend beads in 500 μ L wash buffer.
4. Sediment the beads by centrifugation at 2,500x g for 5 min at +4°C and discard supernatant.
5. Repeat this step at least twice.
6. During the last washing step, transfer the beads to a new tube.

Optional: To increase stringency of the wash buffer, test various salt concentrations e.g. 150 mM – 500 mM, and/or add a non-ionic detergent e.g. Tween 20 or Triton™ X-100.

Elution with 2x SDS-sample buffer

1. Remove the remaining supernatant.
2. Resuspend beads in 80 μ L 2x SDS-sample buffer.
3. Boil beads for 5 min at +95°C to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500x g for 2 min at +4°C.
5. Analyze the supernatant in SDS-PAGE and Western blot.

As an alternative to sample analysis by SDS-PAGE/WB bound proteins be investigated by mass spectrometry after [on-bead digestion](#).