GFP Nanoselector Agarose Kit

HAK21003

Isotype:	Alpaca Recombinant monolconal antibody, VHH
Applications:	IP
Affinity:	KD<1pM
Specificity/Target:	GFP Smartcapture® beads was tested on eGFP, GFP, tagGFP, YFP, CFP, Venus, Citrine, AcGFP.
Description:	Green fluorescent proteins (GFPs) and variants thereof are widely used to study proteinlocalization and dynamics. For biochemical analysis including mass spectrometry and enzymeactivity measurements these GFP-fusion proteins and their interacting factors can be isolated fast and efficiently by immunoprecipitation using the GFP nanoselector Agarose .GFP nanoselector Agarose utilizes small recombinant alpaca antibody fragments covalently coupled to the surface of Agarose beads.
Bead properties:	Bead size: ~ 90 µm; Storage buffer: 20% EtOH.
Binding capacity:	High binding capacity，10 µL slurry bind about 25 µg of recombinant GFP.
Elution:	SDS sample buffer, 0.2 M glycine pH 2.5. For optimal performance, please consider on-bead assays like on-bead digestion for MS analysis.
Stability and Storage:	Shipped at ambient temperature. Upon receipt store at +4°C. Stable for 1 year. Do not freeze.

Buffer compositions

Buffer	Composition
Lysis buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5% NP40. 0.09% sodium azide.
RIPA buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1% SDS, 1% Triton™ X-100, 1% deoxycholate. 0.09% sodium azide.
5x Dilution buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5 mM EDTA. 0.018% sodium azide.
5x wash buffer	10 mM Tris/Cl pH 7.5, 150 mM NaCl, 0.5% NP40, 0.5 mM EDTA. 0.018% sodium azide.
Elution buffer	200 mM glycine pH 2.5.

Mammalian cell lysis

Note: Harvesting of cells and cell lysis should be performed with ice-cold buffers. We strongly recommend to add protease inhibitors to the Lysis buffer to prevent degradation of your target protein and its binding partners. For one immunoprecipitation reaction, we recommend using ~10^6 - 10^7 cells.

1. Choice of lysis buffer:

* For cytoplasmic proteins, resuspend the cell pellet in 200 µL ice-cold Lysis buffer by pipetting up and down. Supplement Lysis buffer with protease inhibitor cocktail and 1 mM PMSF (not included).

* For nuclear/chromatin proteins, resuspend cell pellet in 200 µL ice-cold RIPA buffer supplemented with DNaseI (f.c. 75-150 Kunitz U/mL), MgCl2 (f.c. 2.5 mM), protease inhibitor cocktail and PMSF (f.c. 1 mM)(not included).

GFP Nanoselector Agarose Protocol

2. Place the tube on ice for 30 min and extensively pipette the suspension every 10 min.

3. Centrifuge cell lysate at 12,000 xg for 10 min at +4℃. Transfer cleared lysate (supernatant) to a pre cooled tube and add 300 µL Dilution buffer supplemented with 1 mM PMSF and protease inhibitor cocktail (not included). If required, save 50 µL of diluted lysate for further analysis (input fraction).

Bead equilibration

1. Resuspend the beads by gently pipetting up and down or by inverting the tube. Do not vortex the beads!
2. Transfer 25 µL of bead slurry into a 1.5 mL reaction tube.
3. Add 500 µL ice-cold Dilution buffer.
4. Sediment the beads by centrifugation at 2,500 xg for 5 min at +4℃.
5. Discard the supernatant.

Protein binding

1. Add diluted lysate to the equilibrated beads.
2. Rotate end-over-end for 1 hour at +4℃.

Washing

1. Sediment the beads by centrifugation at 500 xg for 5 min at +4℃.
2. If required, save 50 µL of supernatant for further analysis (flow-through/non-bound fraction).
3. Discard remaining supernatant.
4. Resuspend beads in 500 µL Wash buffer.
5. Sediment the beads by centrifugation at 2,500 xg for 5 min at +4℃. Discard the remaining supernatant.
6. Repeat this step at least twice.
7. 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. Triton™ X-100.

Elution with 2x SDS-sample buffer

1. Remove the remaining supernatant.
2. Resuspend beads in 80 µL 2x SDS-sample buffer.
3. Boil beads for 5 min at +95℃ to dissociate immunocomplexes from beads.
4. Sediment the beads by centrifugation at 2,500 xg for 2 min at +4℃.
5. Analyze the supernatant in SDS-PAGE.

Elution with Glycine-elution buffer

1. Remove the remaining supernatant.
2. Add 50-100 µL Glycine-elution buffer and constantly pipette up and down for 30 - 60 sec at +4℃.
3. Sediment the beads by centrifugation at 2,500 xg for 5 min at +4℃.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

Images

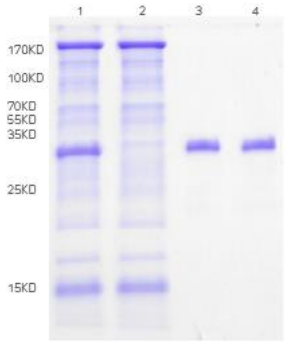
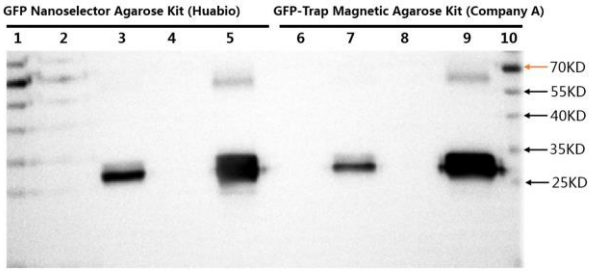


Fig1: Immunoprecipitation of GFP protein with GFP Nanoselector Agarose.

Lane 1: Input
Lane 2: Flow-through
Lane 3: PH=2 Elution
Lane 4: Eluted Beads



Lane 1: Marker
Lane 2: Input (NC)
Lane 3: Input (GFP)
Lane 4: IP (NC)
Lane 5: IP (GFP)

Lane 6: Input (NC)
Lane 7: Input (GFP)
Lane 8: IP (NC)
Lane 9: IP (GFP)
Lane 10: Marker

Fig2: Immunoprecipitate in recombinant fusion of GFP protein with GFP Nanoselector Agarose Kit.