DYKDDDDK tag Nanoselector® Magnetic beads

Code：019-101-003

Introduction

The DYKDDDDK tag Nanoselector® Magnetic beads consists of an anti-DYKDDDDK tag(Flag tag) Nanobody (VHH), which is covalently bound to magnetic beads. DYKDDDDK tag Nanoselector® Magnetic beads is used to immunoprecipitate DYKDDDDK tag-fusion proteins from cell extracts of various organisms like mammals, plants, bacteria, yeast, insects etc. The DYKDDDDK tag Nanoselector® Magnetic beads has great advantages such as high load capacity and no light or heavy chain interference.

Properties

Ligand	Anti-DYKDDDDK tag single domain antibody fragment (VHH, Nanobody)
Reactivity	Specifically binds to DYKDDDDK-tag sequence of the fusion protein.
Binding capacity	20 µg of recombinant DYKDDDDK-fusion protein per 10 µL bead slurry
Bead size	~ 2.8µm
Storage buffer	20 % ethanol
Storage conditions	Upon receipt store at +4°C. **Do not freeze!**
Stability	Stable for 1 year upon receipt.
Shipment	Shipped at low temperature(2-8℃)

Suggested buffer compositions

Required buffer solutions

Buffer	Composition
Lysis buffer	10 mM Tris-HCL pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.5 % NP40
RIPA buffer	10 mM Tris-HCL pH 7.5, 150 mM NaCl, 0.5 mM EDTA, 0.1 % SDS, 1 % TritonX-100, 1 % deoxycholate
Dilution buffer	10 mM Tris-HCL pH 7.5, 150 mM NaCl, 0.5 mM EDTA
Wash buffer	10 mM Tris-HCL pH 7.5, 150 mM NaCl, 0.05 % NP40, 0.5mM EDTA
2xSDS Sample buffer	120 mM Tris-HCL pH 6.8, 20 % glycerol, 4 % SDS, 0.04 % bromophenol blue, 10 % β-mercaptoethanol
Acidic elution buffer	200 mM glycine pH 2.5
Neutralization buffer	1 M Tris-HCL pH 10.4

Note: Use your equivalent cell lysis buffer for other cell types like yeast, plants, insects, bacteria.

Note: Consider using a Wash buf er without detergent for co-immunoprecipitation.

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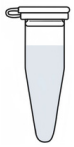
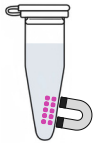
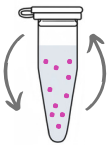
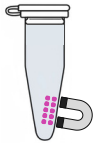
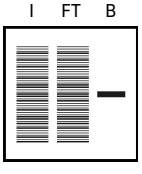
Product sizes

Product	Size
DYKDDDDK tag Nanoselector® Magnetic beads	20 reactions (500 µL slurry)
	200 reactions (5 mL slurry)

Protocol at a glance

General		• Perform all steps at +4°. • Use your preferred cell lysis buffer and cell lysis conditions.
Cell Lysis		• Use 10^6-10^7 cells and 200 µL Lysis buffer. • Perform cell lysis and clear lysate. • Mix 200 µL cleared lysate with 300 µL Dilution buffer.
Bead equilibration		• Transfer 25 µL bead slurry into a 1.5 mL tube. • Equilibrate beads 3x with 500 µL Dilution Buffer.
Protein binding		• Add 500 µL diluted lysate to beads. • Rotate end-over-end for 1 hour at +4°C.
Washing		• Wash beads 3x with 500 µL Wash buffer. • Transfer beads to a new tube during the last washing step.
Elution with SDS-sample buffer		• Resuspend beads in 80 µL 2x SDS-sample buffer. • Boil beads for 10-15 min at +95°C. • Analyze the supernatant in SDS-PAGE / Western Blot.

Immunoprecipitation protocol

Cell material

The following protocol describes the preparation of mammalian cell lysate! For other type of cells, we recommend using 500 µg of cell extract and start the protocol with step Bead equilibration.

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 cell.

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).

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

3. Centrifuge cell lysate at 17,000x g for 10 min at +4°C. Transfer cleared lysate (supernatant) to a precooled 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).

Plant tissue lysis (e.g. Arabidopsis thaliana):

1. Use a tissue to absorb the liquid cultured Arabidopsis seedlings, weigh 0.7 grams, and transfer them to a mortar.
2. Freeze with liquid nitrogen and grind into fine powder with a pestle.
3. Add 4ml of extraction buffer (PBS, 0.5% Triton X-100, 0.5 mM PMSF, protease inhibitor), slowly thaw, and further grind the sample.
4. Transfer the sample to a 1.5 ml microcentrifuge tube and centrifuge at 16000 x g for 20 minutes at 4 ° C.
5. Pass the supernatant through a 0.20 µ m filter syringe and place the extract on ice. 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. Separate the beads with a magnet until the supernatant is clear. Discard the supernatant.

DYKDDDDK tag Nanoselector® Magnetic beads

Code：016-101-003

Protein binding

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

Washing

1. Separate the beads with a magnet until the supernatant is clear.
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. Separate the beads with a magnet until the supernatant is clear. 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. 150-500 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 10-15 min at +95°C to dissociate immunocomplexes from beads.
4. Separate the beads with a magnet.
5. Analyze the supernatant in SDS-PAGE / Western Blot.

Elution with Acidic elution buffer

1. Remove the remaining supernatant.
2. Add 50-100 µL Acidic elution buffer and constantly pipette up and down for 30-60 sec at +4°C or room temperature.
3. Separate the beads with a magnet until the supernatant is clear.
4. Transfer the supernatant to a new tube.
5. Immediately neutralize the eluate fraction with 5-10 µL Neutralization buffer.
6. Repeat this step at least once to increase elution efficiency.

Note: Elution at room temperature is more efficient than elution at +4°C. Prewarm buffers for elution at room temperature.

DYKDDDDK tag(Flag tag) related products

Code Number	Product Description	Applications	Size
016-303-001	Anti-DYKDDDDK tag, AlpHcAbs® Mouse IgG2b antibody	WB,ICC/IF,ELISA,IP,Flow Cyt	100μg
016-303-005	Anti-DYKDDDDK tag, AlpHcAbs® Mouse antibody(HRP)	WB,ELISA	100μg
016-203-001	Anti-DYKDDDDK tag, AlpHcAbs® Rabbit antibody	WB,ICC/IF,ELISA,IP,Flow Cyt	100μg
016-323-001	Anti-DYKDDDDK tag, AlpHcAbs® Mouse IgG1 antibody	WB,ICC/IF,ELISA,IP,Flow Cyt	100μg
016-313-001	Anti-DYKDDDDK tag, AlpHcAbs® Mouse IgG2a antibody	WB,ICC/IF,ELISA,IP,Flow Cyt	100μg
016-303-007	Anti-DYKDDDDK tag, AlpHcAbs® Mouse IgG2b antibody(iFluor488)	WB,ICC/IF,ELISA,Flow Cyt	100μg
016-303-008	Anti-DYKDDDDK tag, AlpHcAbs® Mouse IgG2b antibody(iFluor594)	WB,ICC/IF,ELISA,Flow Cyt	100μg
016-303-009	Anti-DYKDDDDK tag, AlpHcAbs® Mouse IgG2b antibody(iFluor647)	WB,ICC/IF,ELISA,Flow Cyt	100μg
016-503-001	Anti-DYKDDDDK tag, AlpHcAbs® Human antibody	WB,ELISA,IP,ICC/IF,Flow Cyt	100μg

Nanoselector related products

Code Number	Product Description	Applications	Size
002-101-002	Myc tag Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
002-101-003	Myc tag Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
019-101-002	GFP Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
019-101-003	GFP Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
016-101-002	DYKDDDDK tag Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
016-101-003	DYKDDDDK tag Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
003-101-002	HA tag Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
003-101-003	HA tag Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
015-101-002	MBP Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
015-101-003	MBP Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
010-101-002	GST Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
010-101-003	GST Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
011-101-002	SNAP tag Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
012-101-002	Halo Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
013-101-002	mNeongreen Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
014-101-002	TurboGFP Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
004-101-002	c-His tag Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
004-101-003	c-His tag Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)

Nanoselector related products

Code Number	Product Description	Applications	Size
049-101-002	mWasabi Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
017-101-002	TagFP Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
025-101-002	Rabbit IgG Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
025-101-003	Rabbit IgG Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
001-101-002	Mouse IgG Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
001-101-003	Mouse IgG Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
001-300-002	Mouse IgG&Rabbit IgG Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
001-300-003	Mouse IgG&Rabbit IgG Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
067-101-003	Streptavidin Magnetic beads	IP,CHIP,MS,Purification	1mL(40 rxns)
064-101-002	V5 tag Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
064-101-003	V5 tag Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
086-101-003	Spycatcher Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
086-101-002	Spycatcher Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
082-101-002	StayGold/mBaojin Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
082-101-003	StayGold/mBaojin Nanoselector Magnetic	IP,CHIP,MS,Purification	0.5mL(20 rxns)
020-101-002	RFP Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
020-101-003	RFP Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
023-101-003	Human IgG Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
026-101-002	tdTomato Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
026-101-003	tdTomato Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
028-101-002	mScarlet Nanoselector Agarose	IP,CHIP,MS,Purification	0.5mL(20 rxns)
028-101-003	mScarlet Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
078-101-003	Biotin Nanoselector Magnetic beads	IP,CHIP,MS,Purification	0.5mL(20 rxns)
100-100-100	Binding Control Nanoselector Agarose	IP,CHIP,MS,Purification	1mL(40 rxns)
100-100-200	Binding Control Magnetic beads	IP,CHIP,MS,Purification	1mL(40 rxns)