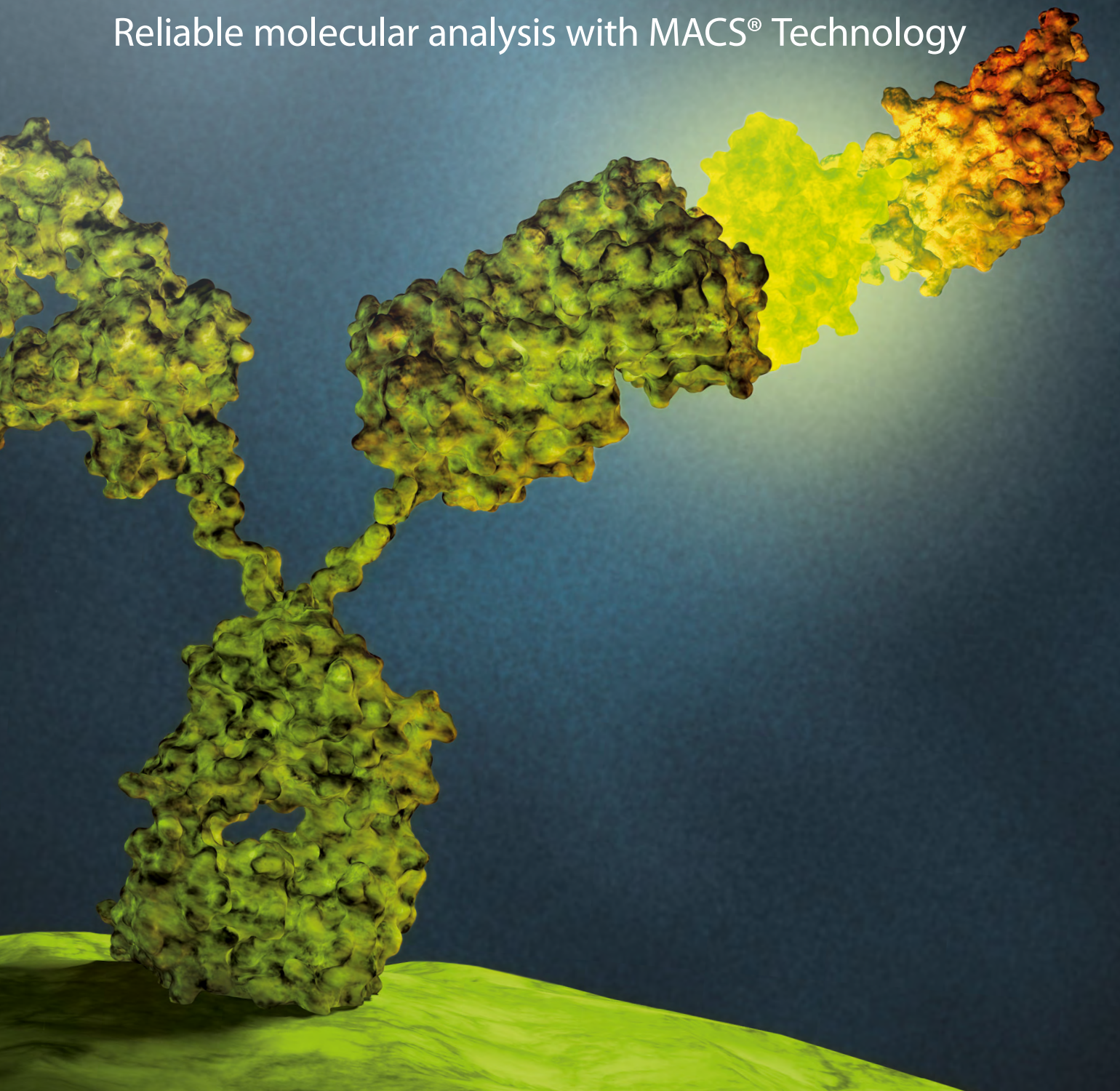
Miltenyi Biotec

Fast and sensitive protein isolation

Reliable molecular analysis with MACS® Technology



MACS Technology

for molecular applications

Products for fast and sensitive protein isolation

Proteomic analyses are conducted in many research fields to gain a better understanding of particular biological processes and play a major role in drug screening and target identification procedures.

MACS Technology for protein isolation is simple, straightforward, and takes you from cell or tissue sample to pure protein in less than two hours.

One technology for several applications

- Isolation of epitope-tagged proteins
- (Co)-Immunoprecipitations of proteins
- Isolations of proteins via biotinylated capture probes

Contents

How MACS Technology works

Isolate proteins quickly and sensitively

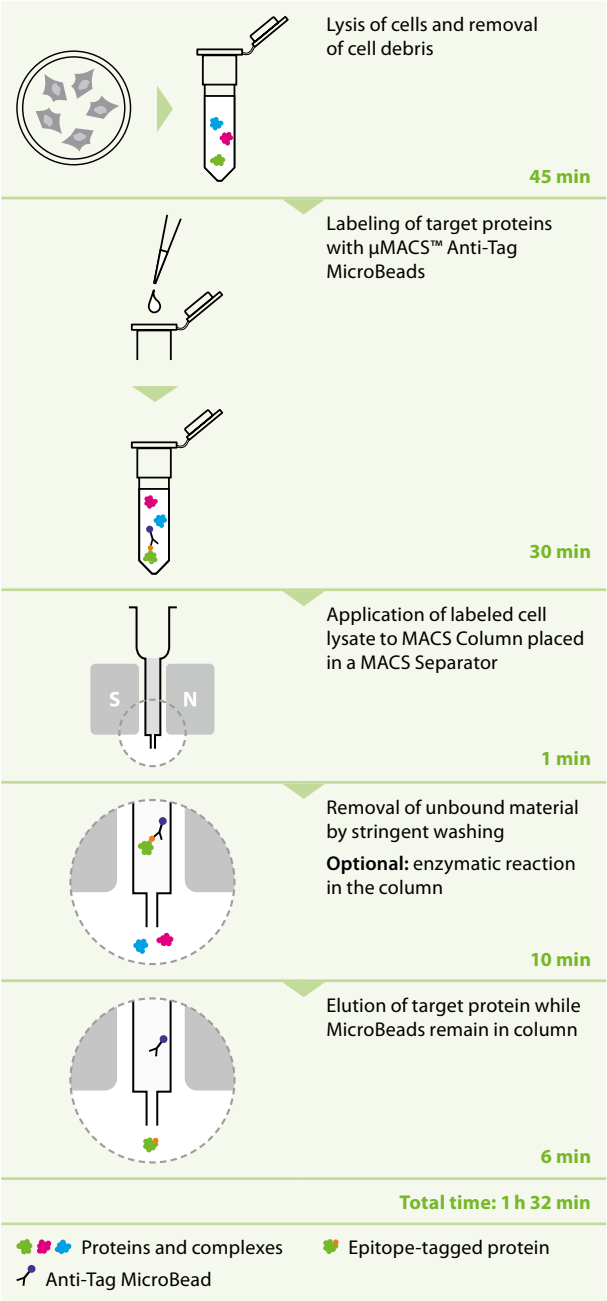


Figure 1: Principle of MACS Technology for isolation of epitope-tagged proteins.

The operating principle

Superparamagnetic µMACS MicroBeads are applied to the cell lysate and instantly bind to their target protein. The magnetically labeled protein is then isolated and purified in a MACS Column positioned within the magnetic field of a MACS Separator. After thorough washing, pure protein is eluted.

How you benefit from MACS Technology for protein isolation

High sensitivity
Even rare proteins can be isolated.

High Speed
Less than two hours from cells or tissue to pure protein.

High specificity
Low background binding.

High recovery
No loss of material due to centrifugation or buffer removal.

Reproducibility and reliability
Protein isolation procedures can be easily automated.

Did you know?

Instead of eluting the protein, enzymatic reactions such as kinase assays can be performed directly in the column. For incubation at elevated temperatures, the thermoMACS™ Separator is available.

Tools for success

µMACS MicroBeads

- Superparamagnetic – only magnetized in a magnetic field
- Small in size – just 50 nm in diameter
- Non-sedimenting with extremely high reaction kinetics – instantly bind target protein
- Low nonspecific binding

MACS Column

- Packed with steel spheres that enhance the magnetic field
- Buffers run by gravity flow, so there is no need for centrifugation or buffer removal, thus preventing a loss of target
- Thorough rinsing procedure

MACS Separators

The **µMACS Separator** is a permanent magnet for manual processing of up to four samples in parallel. For higher throughput experiments, the procedure can easily be scaled up to a 96-well format utilizing the **MultiMACS M96 Separator.**
An automated process is achieved by integrating this benchtop instrument into a robotic pipetting system.

Figure 2: MACS Columns enable fast and sensitive separations.

Figure 3: MultiMACS M96 Separator

Figure 4: µMACS Separator

Epitope-tagged protein isolation

Sensitive isolation at high speed

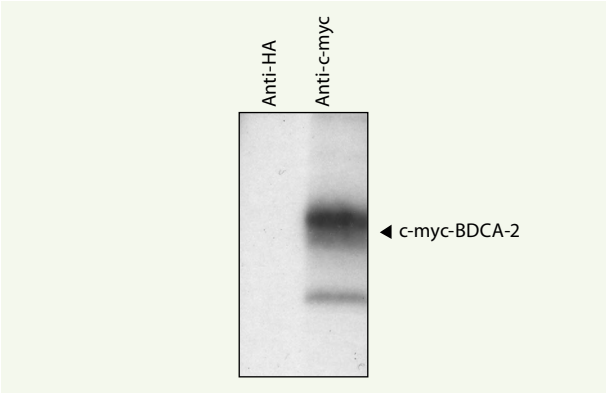


Figure 5: Specific isolation of recombinant fusion protein with µMACS Anti-c-myc MicroBeads. Cells were transfected with a vector encoding c-myc-BDCA-2 and labeled with 35S-methionine. The recombinant c-myc-BDCA-2 protein was purified with µMACS Anti-c-myc MicroBeads. Left lane: control purification with Anti-HA MicroBeads. The figure shows an autoradiogram after SDS-PAGE.

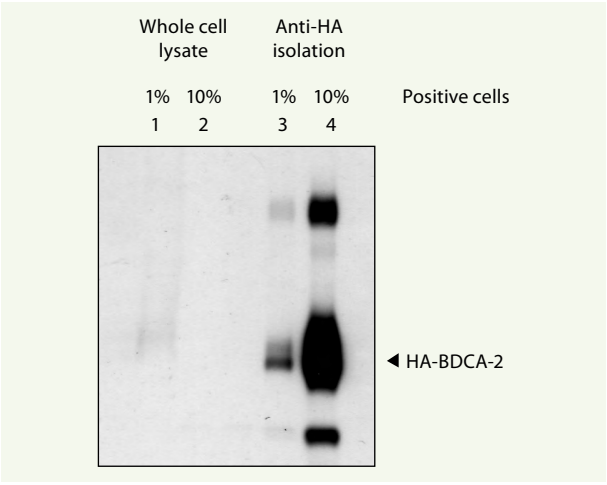


Figure 6: Sensitive isolation of recombinant fusion protein with µMACS Anti-HA MicroBeads. 10^7 mouse pre-B cells (1881) were transfected with a vector encoding HA-tagged BDCA-2, and protein isolation was performed using cell populations with either 1% (lane 1, 3) or 10% (lane 2, 4) positively transfected cells. Whole cell lysates (lane 1, 2) or 20% of the protein isolation eluate with µMACS Anti-HA MicroBeads (lane 3, 4) were separated by SDS-PAGE, blotted on a membrane, and detected by using an Anti-HA-HRP antibody.

Isolation of epitope-tagged proteins

The **µMACS Tag Isolation Kits** contain µMACS MicroBeads coupled to high-quality monoclonal antibodies specific for proteins tagged with:

- HA (hemagglutinin)
- His (histidine-epitope)
- DYKDDDDK (also known as FLAG® tag)
- c-myc
- GFP (green fluorescent protein)
- GST (glutathione S-transferase)

These µMACS Anti-Tag MicroBeads are optimized for the specific and sensitive isolation of recombinant epitope-tagged proteins. Particularly when working with eukaryotic cells, sensitive isolation with low rate of background binding is essential.

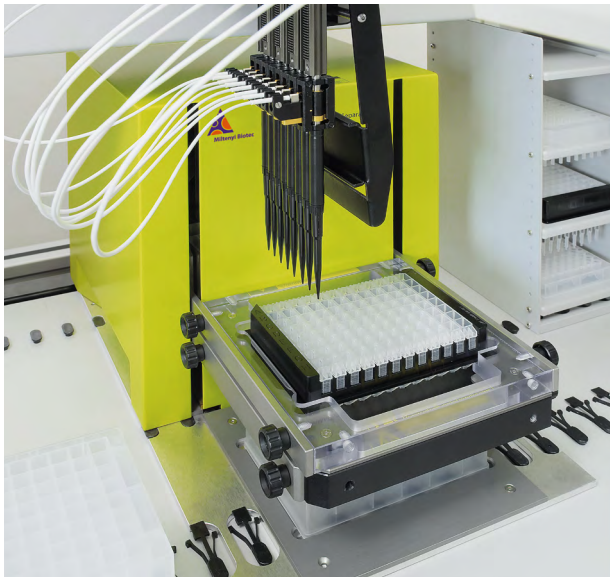


Figure 7: MultiMACS M96 Separator integrated in a pipetting robot for automated protein isolation in a 96-well format.

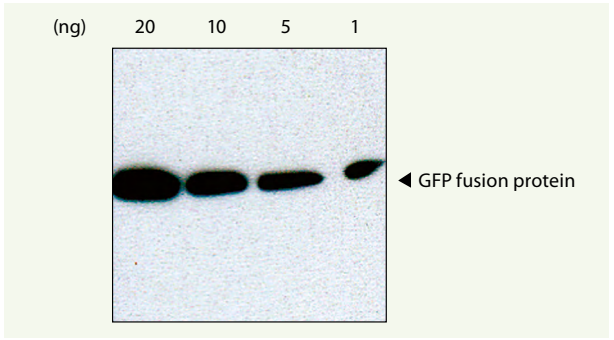


Figure 8: Sensitive detection of recombinant fusion protein with Anti-GFP-HRP antibody. 20 ng, 10 ng, 5 ng, and 1 ng GFP fusion protein were separated by SDS-PAGE and blotted on PVDF membrane. GFP fusion proteins were detected with Anti-GFP-HRP antibody (1:5,000, 1 hour, room temperature) and ECL reagent (GE Healthcare).

Automated 96-well isolation of epitope-tagged proteins in less than two hours

The **MultiMACS Epitope tag isolation kits** enable researchers to magnetically isolate c-myc-, GFP-, GST-, HA-, His-, or DYKDDDDK-tagged proteins in a higher throughput procedure.

Up to 96 samples can be processed in parallel with the compact, benchtop MultiMACS M96 Separator, either automated or manually when integrated in a pipetting robot.

Analysis of epitope-tagged proteins

Monoclonal Anti-c-myc, Anti-GFP, Anti-HA, Anti-His, or Anti-DYKDDDDK-tagged antibodies are available with several conjugates:

- Biotin
- HRP
- FITC
- PE

Fluorochrome-conjugated Anti-Tag antibodies allow immunofluorescence analysis by flow cytometry or fluorescence microscopy. Biotinylated antibodies can be used in combination with streptavidin conjugates, such as streptavidin-HRP, in Western blot detection, or with fluorochrome-streptavidin for immunofluorescence analysis.

Directly coupled to horseradish peroxidase (HRP), the antibodies simplify Western blot or ELISA analysis because incubations with secondary antibodies are unnecessary.

The antibodies are ideally suited for

- flow cytometry
- fluorescence microscopy
- immunoblotting / Western blotting
- ELISA

MACS Protein A/G MicroBeads

Immunoprecipitation of proteins

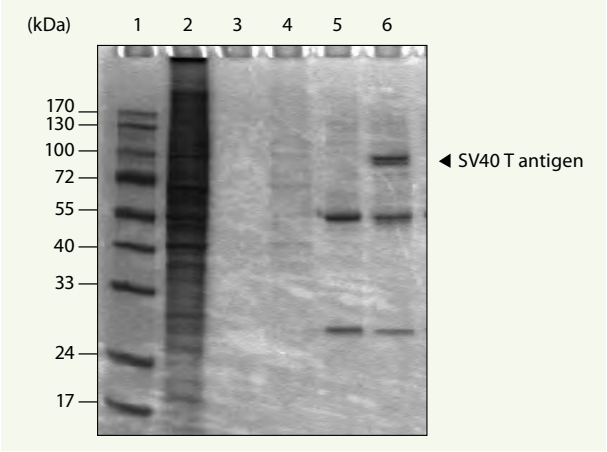


Figure 9: Immunoprecipitation of the SV40 large T antigen Immunoprecipitation was performed from COS-7 cells using µMACS Protein G MicroBeads. Coomassie-stained gradient SDS-PAGE of a protein marker (lane 1), the flow through (lane 2), the wash fraction (lane 4), the immuno-precipitated large T antigen (lane 6, indicated by the arrow), and an isotype-matched control antibody (lane 5).

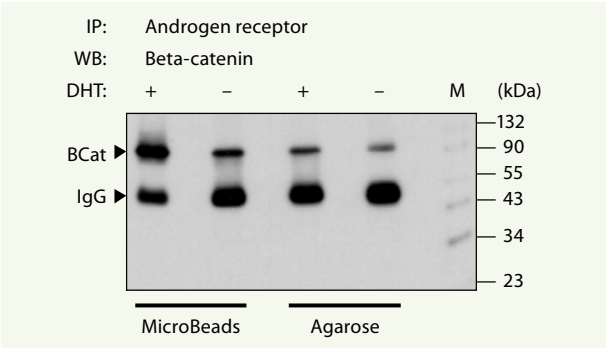


Figure 10: Co-immunoprecipitation of Beta-Catenin Androgen receptor was immunoprecipitated from dihydrotestosterone (DHT)-stimulated (lanes 1, 3) or unstimulated (lanes 2, 4) LNCaP cells with µMACS Protein G MicroBeads (lanes 1, 2) or with Protein A/G agarose beads (lanes 3, 4). Western blot using anti-beta-catenin antibody shows beta-catenin (BCat) co-immunoprecipitated with androgen receptor. (Courtesy of D. Mulholland, Vancouver, Canada)

Immunoprecipitation with µMACS Protein A/G MicroBeads

The **µMACS Protein A/G MicroBeads** were developed for small-scale analytic immunoprecipitation (IP). The extremely small µMACS Protein A/G MicroBeads ensure fast reaction kinetics. Formation of the labeled immune complex is generally completed in 30 minutes. There is no need for overnight incubation.

High sensitivity
Due to the small size of µMACS MicroBeads, binding to target proteins is extremely fast and efficient; high amounts of target protein can be captured per sample.

High speed
MACS Technology saves time. The experiment can be completed within 2 hours, while conventional IP may require up to one day of work.

High specificity
The minimized non-specific binding of µMACS Protein A/G MicroBeads and the efficient and gentle washing in the column significantly reduces background binding. The washing procedure can be optimized for any target molecule, and even fragile protein complexes can be successfully isolated by co-IP with MACS Technology.

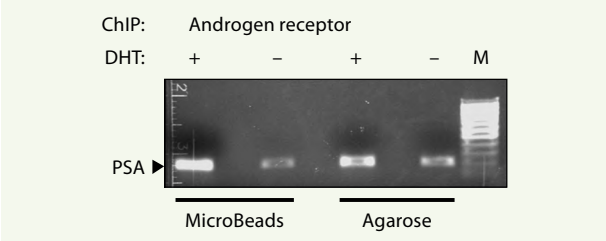


Figure 11: Chromatin immunoprecipitation (ChIP) PCR reaction of the prostate-specific antigen (PSA) gene using DNA obtained by ChIP from cultured cells. Immunoprecipitation was carried out with anti-androgen-receptor antibody and µMACS Protein G MicroBeads (lanes 1, 2) or with Protein A/G agarose beads (lanes 3, 4). Dihydrotestosterone (DHT)-stimulated (lanes 1, 3) or unstimulated (lanes 2, 4) cells were used for ChIP. (Courtesy of D. Mulholland, Vancouver, Canada)

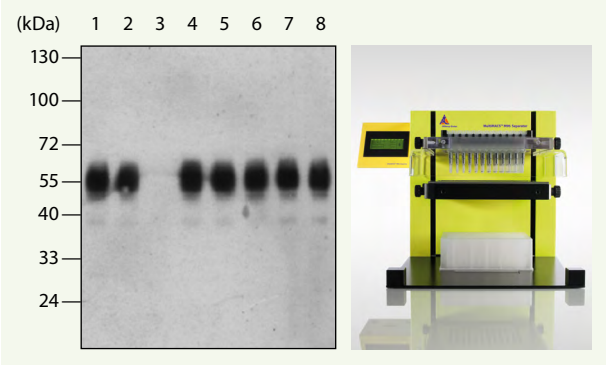


Figure 12: Isolation of mouse PD-1 protein with MultiMACS Protein G MicroBeads Cell lysates of 106 CHO cells, lane 3, and CHO cells expressing c-myc-tagged mouse PD-1 tagged with c-myc, lane 1, 2, 4–8, were immunopurified using an anti-c-myc horseradish peroxidase conjugate and MultiMACS Protein G MicroBeads on a Multi-8 Column strip. Purified eluates were analyzed via SDS PAGE and subsequent immunoblotting using anti-c-myc antibodies.

ChIP-in-a-column with MACS Technology

The **µMACS Protein A/G MicroBeads** improve standard immunoprecipitation and significantly accelerate the search for interacting proteins. Chromatin immunoprecipitation (ChIP) protocols also benefit from the higher specificity and lower background binding of µMACS Protein A/G MicroBeads.

Automated 96-well immunoprecipitation or ChIP

The **MultiMACS M96 Separator** allows the parallel processing of up to 96 samples with Multi-8 or Multi-96 Columns. This compact benchtop instrument allows semi-automated or – in combination with a robotic pipetting system – automated magnetic isolation of molecules.

Both immunoprecipitation and ChIP can easily be upgraded for the automated processing of up to 96 samples in parallel using the **MultiMACS Protein A/G Kits.**

MACS Streptavidin Kit

Isolation of biotinylated molecules

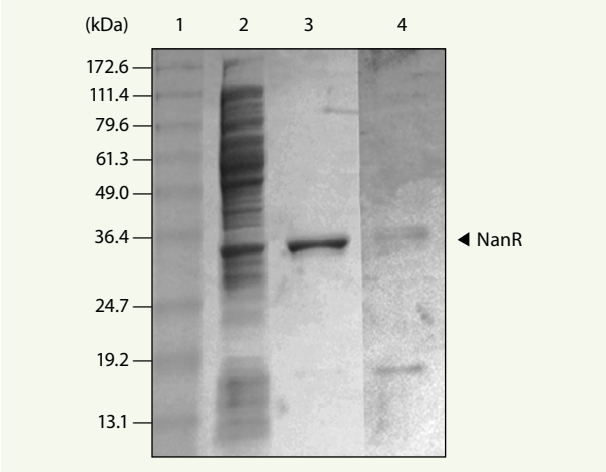


Figure 13: Purification of NanR Lanes show Coomassie-stained samples fractionated by 4–20% SDS-PAGE. Lane 1: Benchmark Prestained Markers (Invitrogen); lane 2: soluble protein fraction from JM109 harboring pSX675 after induction with 0.2% L-arabinose (30.9 µg); lane 3: purified recombinant NanR (3.5 µg); lane 4: NanR from *E. coli* MC4100 (1.68 µg).

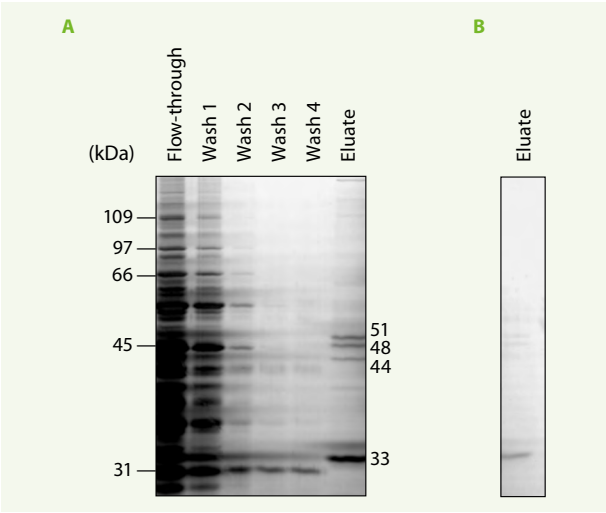


Figure 14: Isolation of specific RNA binding proteins. Yeast crude extract was pre-cleared and subsequently incubated with a full-length Mating Factor A2 mRNA bound to a 3'-biotinylated complementary single-stranded oligonucleotide and magnetically labeled with µMACS Streptavidin MicroBeads. The figure (A) shows the silver-stained SDS gel. Four proteins with molecular weights of 33, 44, 48, and 51 kDa, which bind specifically to the RNA sequence, were isolated (eluate). As a control, a magnetically labeled mutant mRNA lacking the binding site for Mating Factor A2 binding proteins was used. In the control experiment (B) no specific proteins were isolated. (Courtesy of Dr. A. Albig, Washington State University, USA)

Isolation of biotinylated molecules

The **µMACS Streptavidin Kit** specifically isolates any molecule interacting with a biotinylated capture probe. This is useful for searching and analyzing binding partners of biotinylated proteins, such as receptor ligands or signalling activators. The principle also works for nucleic acids, including mRNA or viral sequences. Even large molecular complexes, organelles, or viable viruses can be purified with MACS Technology.

Applications for the µMACS Streptavidin Kit

- Detection and analysis of:
  - protein-protein interaction
  - DNA-protein interaction
  - RNA-protein interaction
- Immunoprecipitation using biotinylated antibodies
- Specific transcripts isolation
- microRNAs isolation
- tRNA molecule isolation
- Ribozyme isolation
- Virus isolation
- Serial analysis of gene expression (SAGE)
- Subtractive hybridization
- Phage and yeast display

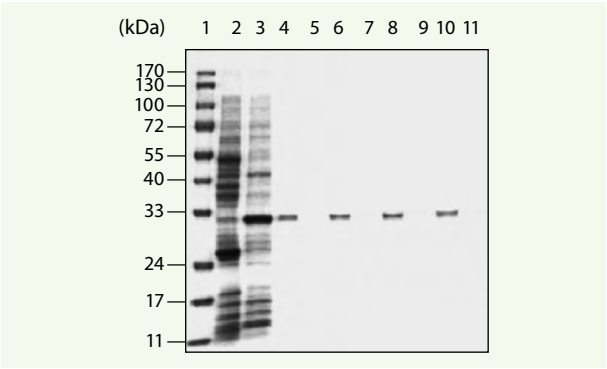


Figure 15: Isolation of HA-tagged proteins with MultiMACS Streptavidin MicroBeads. 100 µL lysates of non-transfected *E. coli* (control lysate) and *E. coli* transfected with an expression vector for HA-tagged protein (test lysate) were incubated with 2 µg anti-HA-Biotin and 100 µL of Streptavidin MicroBeads for 30 minutes on ice. The proteins were then purified with a MultiMACS M96 Separator and analyzed on an SDS gel stained with Coomassie Brilliant Blue. Lane 1: molecular weight marker; lane 2: *E. coli* control lysate; lane 3: transfected *E. coli* lysate; lanes 4, 6, 8, 10: purified test lysate; lanes 5, 7, 9, 11: purified control lysate.

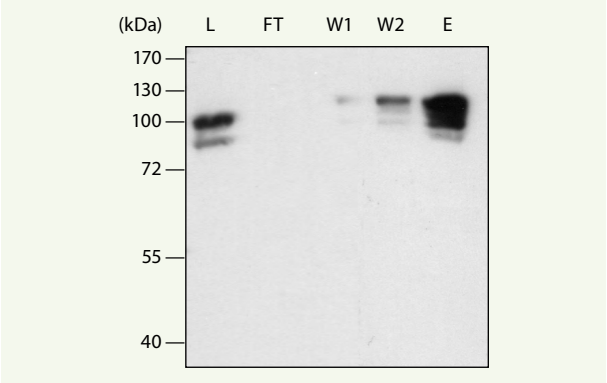


Figure 16: Isolation of phosphorylated STAT-3 (pSTAT-3) from activated T cells. T cells from human PBMCs were activated and expanded in the presence of IL-2 using the T Cell Activation/Expansion Kit (# 130-091-441). After three days, cells were incubated with IL-15 for 15 minutes, washed, and lysed with Cell Lysis Buffer. Whole cell lysate was incubated with a biotinylated 31-bp DNA probe comprising the STAT-3 binding sequence. µMACS Streptavidin MicroBeads were added, and magnetic isolation was performed. The transcription factor was eluted with high salt Elution Buffer. The Western blot (using a phosphospecific STAT-3 antibody) shows a high amount of pSTAT-3 in the eluate. L: cell lysate; FT: flow-through; W1, W2: wash fractions; E: eluate.

Automated 96-well isolation of biotinylated probes

The **MultiMACS Streptavidin Kits** in combination with the MultiMACS M96 Separator simultaneously isolate up to 96 samples in an automated process when used in combination with a robotic pipetting system.

MACSflex MicroBead Kits

Fast coupling to biomolecules

MACSflex MicroBead Kits

Microbeads for flexible coupling of any biomolecule of choice

The MACSflex MicroBeads offer an NHS-activated surface for flexible and fast covalent coupling of a variety of biomolecules (e.g. antibodies, proteins, oligonucleotides, peptides) without additional reagents or time-consuming steps. The fast coupling procedure allows you to use the coupled MicroBeads in downstream applications, such as epitope-tagged protein isolations and organelle isolations, within the same working day.

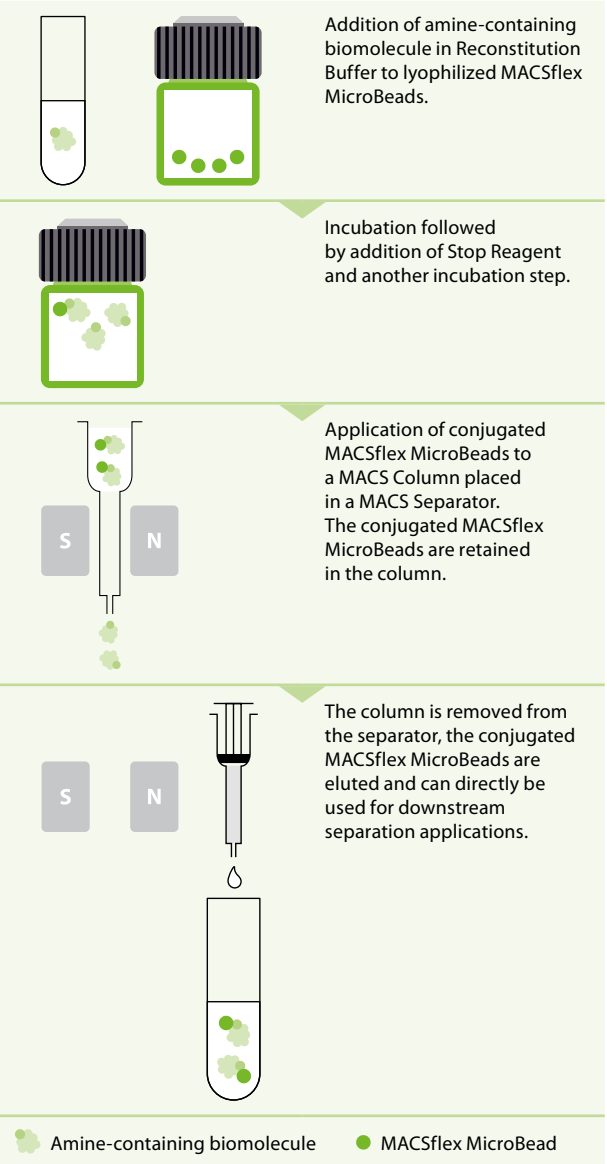


Figure 17: Principle of biomolecule-coupling to MACSflex MicroBeads.

Product information

Product	Order no.
Isolation of epitope-tagged proteins	
µMACS c-myc Isolation Kit 40 isolations	130-091-123
µMACS Anti-c-myc Starting Kit 40 isolations	130-091-284
µMACS His Isolation Kit 40 isolations	130-091-124
µMACS Anti-His Starting Kit 40 isolations	130-091-285
µMACS HA Isolation Kit 40 isolations	130-091-122
µMACS Anti-HA Starting Kit 40 isolations	130-091-286
µMACS GFP Isolation Kit 40 isolations	130-091-125
µMACS Anti-GFP Starting Kit 40 isolations	130-091-288
µMACS GST Isolation Kit 40 isolations	130-091-370
µMACS Anti-GST Starting Kit 40 isolations	130-091-493
µMACS DYKDDDDK Isolation Kit 40 isolations	130-101-591
µMACS Anti-DYKDDDDK Starting Kit 40 isolations	130-101-636
96-well isolation of epitope-tagged proteins	
MultiMACS c-myc Isolation Kit (12×8)	130-094-250
MultiMACS c-myc Isolation Kit (4×96)	130-094-251
MultiMACS GFP Isolation Kit (12×8)	130-094-252
MultiMACS GFP Isolation Kit (4×96)	130-094-253
MultiMACS GST Isolation Kit (12×8)	130-094-254
MultiMACS GST Isolation Kit (4×96)	130-094-256
MultiMACS HA Isolation Kit (12×8)	130-094-255
MultiMACS HA Isolation Kit (4×96)	130-094-257
MultiMACS His Isolation Kit (12×8)	130-094-258
MultiMACS His Isolation Kit (4×96)	130-094-259
MultiMACS DYKDDDDK Isolation Kit (12×8)	130-101-621
MultiMACS DYKDDDDK Isolation Kit (4×96)	130-101-623

Product	Order no.
Immunoprecipitation	
µMACS Protein A MicroBeads 2 mL, 40 isolations	130-071-001
µMACS Protein G MicroBeads 2 mL, 40 isolations	130-071-101
µMACS Protein A/G Starting Kit 1 µMACS Separator, 20 µ Columns, 1 MACS Multistand, 40 isolations	130-042-601
MultiMACS Protein A Kit (24×8) with sealing foil; 2 Microtiter Plates, U-bottom, 192 isolations	130-092-944
MultiMACS Protein G Kit (24×8) 5× foil; 2 Microtiter Plates, U-bottom, 192 isolations	130-092-946
MultiMACS Protein A Kit (4×96) 384 isolations	130-092-945
MultiMACS Protein G Kit (4×96) 384 isolations	130-092-947
Isolation via biotinylated molecules and MACSflex MicroBead Kits	
µMACS Streptavidin Kit 20 isolations	130-074-101
µMACS Streptavidin Starting Kit 20 isolations	130-091-287
MultiMACS Streptavidin Kit (12×8) 96 isolations	130-092-948
MultiMACS Streptavidin Kit (4×96) 384 isolations	130-092-949
MACSflex MicroBead Kit	130-105-805
MACSflex MicroBead Kit	130-105-806
MACSflex MicroBead Kit	130-105-809
MACS Separators and MACS Columns for molecular analysis	
µMACS Separation Unit Compatible with µ Columns	130-042-602
thermoMACS Separation Unit Compatible with µ Columns	130-091-136
MiniMACS Separation Unit Compatible with M Columns	130-042-102
OctoMACS Separation Unit Compatible with M Columns	130-042-109
MultiMACS M96 Separator Compatible with Multi-8 and Multi-96 Columns	130-091-937
µ Column 20 columns	130-042-701
M Column 10 columns	130-042-801
Multi-8 Columns, molecular (12×8) 12 Multi-8 Columns, 1 MultiColumn Frame, 1 Deep Well Block, 1 Microtiter Plate	130-092-444
Multi-96 Column, molecular (4×96) 4 Multi-96 Columns with MultiColumn Frames, 4 Deep Well Blocks, 4 Microtiter Plates	130-092-445

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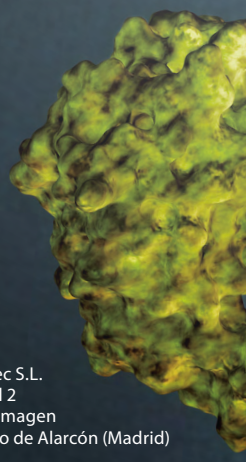

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