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MultiMACS™ Epitope Tag Isolation Kits

MultiMACS™ c-myc Isolation Kit (12×8)	130-094-250
MultiMACS c-myc Isolation Kit (4×96)	130-094-251
MultiMACS His Isolation Kit (12×8)	130-094-258
MultiMACS His Isolation Kit (4×96)	130-094-259
MultiMACS HA Isolation Kit (12×8)	130-094-255
MultiMACS HA Isolation Kit (4×96)	130-094-257
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

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1. Description

1.1 Components and size

MultiMACS™ Epitope Tag Isolation Kits (12×8)	Order no.
μMACS™ Anti-c-myc MicroBeads	130-094-250
μMACS Anti-His MicroBeads	130-094-258
μMACS Anti-HA MicroBeads	130-094-255
μMACS Anti-GFP MicroBeads	130-094-252
μMACS Anti-GST MicroBeads	130-094-254

Components	3×2 mL of μMACS Anti-Tag MicroBeads
	1×50 mL of Equilibration Buffer
	Multi-8 Column Box containing:
	12×Multi-8 Columns, separately packaged
	1 MultiColumn Frame
	1 Deep Well Block, sealed, 96×2.5 mL
	1 Microtiter Plate, U-bottom, with adhesive sealing foil

The cover photo shows a replica of the DNA model built in 1953 by James D. Watson and Francis Crick at the Cavendish Laboratory in Cambridge. This model is located at Heureka, the Finnish Science Centre. Photography by Alexander Budde; © Miltenyi Biotec GmbH, Germany. Detailed information on the history of the Watson-Crick model can be found in: de Chadarevian, S. (2003) Relics, replicas and commemorations. Endeavour 27: 75–79.

Size For 96 reactions, each for the isolation of up to 20 pmol protein (e.g. equivalent to 0.5–1 µg of a 50 kDa protein)

MultiMACS™ Epitope Tag Isolation Kits (4×96)	Order no.
µMACS™ Anti-c-myc MicroBeads	130-094-251
µMACS Anti-His MicroBeads	130-094-259
µMACS Anti-HA MicroBeads	130-094-257
µMACS Anti-GFP MicroBeads	130-094-253
µMACS Anti-GST MicroBeads	130-094-256

Components 5×4.6 mL of µMACS Anti-Tag MicroBeads
1×50 mL of Equilibration Buffer
Multi-96 Column Box containing:
4×Multi-96 Columns, each packaged sterile in a Deep Well Block, 96×2.5 mL
4 Microtiter Plates, U-bottom, with adhesive sealing foil

Size For 384 reactions, each for the isolation of up to 20 pmol protein (e.g. equivalent to 0.5–1 µg of a 50 kDa protein)

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Product format Anti-Tag MicroBeads are supplied in a solution containing 0.05% sodium azide.

Storage Store µMACS Anti-Tag MicroBeads protected from light at 2–8 °C. Do not freeze. The expiration dates are indicated on the vial labels. Store Columns, Deep Well Blocks, and Microtiter Plates at room temperature, dry, and protected from light.

1.2 MACS® Technology for epitope-tagged protein isolation

Frequently, protein-specific monoclonal antibodies are not available for an emerging research project regarding the function of a certain protein. Adding a so-called epitope tag, an easily detectable marker, to the protein of interest is a popular method to render the target protein detectable by using an antibody against the epitope tag.^{1,2} The short length of the epitope tag, 6–20 amino acids, and its position at the amino- or carboxyl terminus of the protein, usually prevents interference of the tag with the protein function. Alternatively, target proteins can be fused to a second full-length protein. These proteins, for example GFP or GST, are also referred to as tags.

To enable researchers to isolate high-purity epitope-tagged proteins in a 96-well format from different sources, Miltenyi Biotec has developed MultiMACS™ Epitope Tag Isolation Kits. Kits are available for the high-throughput purification of the most popular epitope tags c-myc, His, HA, GFP, and GST. The optimized procedure takes advantage of MACS® Technology (fig. 1): In a first step, superparamagnetic, 50 nm-sized MicroBeads coupled with tag-specific, monoclonal antibodies, are added



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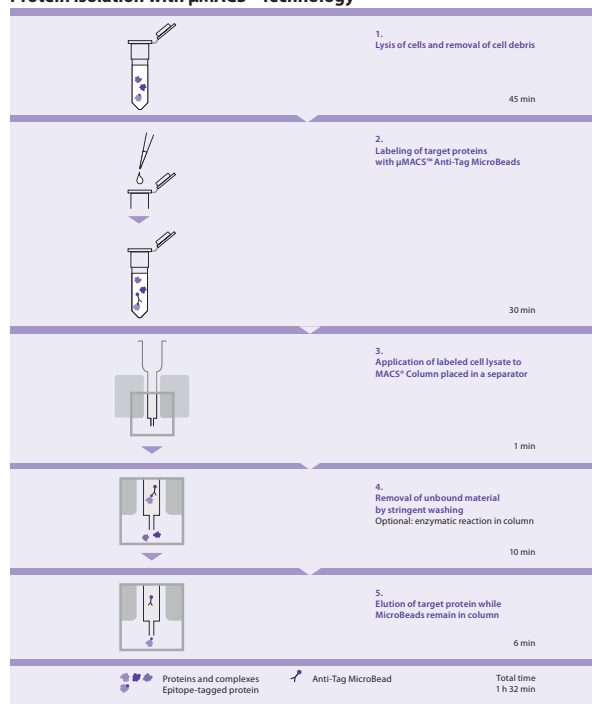
to the lysed cells. The MicroBeads bind to the epitope-tagged proteins in the lysate—thus magnetically labeling them. Then, the mixture is transferred to Multi-8/96 Columns and exposed to a strong magnetic field within the MultiMACS 96 Separator. The magnetically labeled target proteins are retained within the column while other cell components flow through. After stringent washing steps, the epitope-tagged proteins can be eluted with high purity and can be used for further analysis such as SDS polyacrylamide gel electrophoresis (SDS-PAGE) or mass spectrometry.³ If downstream experiments require functional proteins, a native elution buffer can be used. Please note that enzymatic assays can also be performed within the column (for details refer to section 5.3).

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Protein isolation with µMACS™ Technology



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1.3 Product applications

μMACS Anti-Tag MicroBeads were developed for the direct analytical immunoprecipitation or for analytical co-immunoprecipitations of proteins from cell or tissue lysates. The immunoprecipitated proteins or protein complexes can be analyzed, for example, by SDS-PAGE or mass spectrometry. Also, consecutive in-column enzymatic reactions can be performed directly on the column.

Product specifications

- μMACS Anti-c-myc MicroBeads
Target sequence: EQKLISEEDL⁴
Tag origin: Human *c-myc* protooncogene
Isotype: mouse IgG1
- μMACS Anti-HA MicroBeads
Target sequence: YPYDVPDYA^{5,6}
Tag origin: Influenza virus hemagglutinin
Isotype: mouse IgG1
- μMACS Anti-His MicroBeads
Target sequence: HHHHHH⁷
Tag origin: synthetic
Isotype: mouse IgG2b
- μMACS Anti-GFP MicroBeads
Target sequence: whole green fluorescent protein (238-residue polypeptide)⁸
Tag origin: *Aequorea victoria* jellyfish
Isotype: mouse IgG1

- μMACS Anti-GST MicroBeads
Target sequence: whole glutathione-S-transferase (220-residue polypeptide)⁹
Tag origin: *Schistosoma japonicum*
Isotype: mouse IgG1

GST and GFP, both full-length proteins, are often used as a protein tags. GFP has the additional property of inherent fluorescence at 400 nm excitation. GFP fusion proteins can therefore be located and studied within living cells by fluorescence microscopy.

1.4 Reagent and instrument requirements

- MultiMACS 96 Separator (# 130-091-937).
- Heating block plus reagent reservoir compatible with 8-well or 96-well (8/96-well) format for heating elution buffer to 95 °C, for example, thermocycler, heating block, or thermoshaker compatible with 8/96-well format.
- For large-scale, complete lysis of cells (including nucleus): Bead mill, for example, Mini-Bead Beater-96, BioSpec Products.
- Stainless steel beads for parallel lysis or homogenization, see supplier of bead mill.

For manual use

- 8-channel pipette with tips, for example, 8-Channel Impact® Pipettor from Matrix Technologies:

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- i) volume range 15–1250 μL with 1250 μL TallTip (102 mm) Filter Tips for transferring lysate and dispensing wash buffers quickly without foaming
- ii) volume range 5–250 μL for dispensing hot elution buffer in a single pipetting mode
- Disposable reagent reservoirs for multichannel pipettes as reservoirs for lysis buffer and wash buffers.

For automated use

- Liquid handling platform with four to eight pipetting channels, range 20 μL to 1 mL, and a gripper tool to grip plates sideways.
- MultiMACS Adapter (please contact technical support).
- Reservoir holders and reagent reservoirs for lysis buffer, wash buffers, and MicroBeads.
- Communication software tool mumcli.exe for control of MultiMACS Separator by PC of automated pipetting platform available for download: www.miltenyibiotec.com.

Recommended buffers

Cell lysis buffer

▲ For recommendations on appropriate use of lysis buffers, see section 3.1, Lysis buffers.

▲ To prevent protein degradation, adding protease inhibitor to the lysis buffer is recommended, for example, from Roche Applied Science; please see section 5.1, Protease inhibitors.

- Triton® X-100 lysis buffer: 150 mM NaCl, 1% Triton X-100, 50 mM Tris HCl (pH 8.0).
- High-salt lysis buffer: 500 mM NaCl, 1% Igepal® CA630 (NP-40), 50 mM Tris HCl (pH 8.0).
- Low-salt lysis buffer: 1% Igepal CA630 (NP-40), 50 mM Tris HCl (pH 8.0).

▲ **Note:** The suitability of a lysis buffer for the experiment has to be determined experimentally. If no previous experience exists, usage of Triton X-100 lysis buffer is recommended.

Wash buffers

- Wash buffer 1: 150 mM NaCl, 1% Igepal CA-630 (formerly NP-40), 0.5% sodium deoxycholate, 0.1% SDS, 50 mM Tris HCl (pH 8.0).
- Wash buffer 2: 20 mM Tris HCl (pH 7.5).

Elution buffer for immediate SDS-PAGE analysis

SDS gel loading buffer (1×): 50 mM Tris HCl (pH 6.8), 50 mM DTT, 1% SDS, 0.005% bromophenol blue, 10% glycerol.

Buffers for in-column enzymatic reaction

Refer to section 5.3, In-column enzymatic reaction.

Buffers for subsequent mass spectrometry analysis

Refer to section 3.2, Buffers for mass spectrometry analysis.

1.5 Related products

- Anti-c-myc-HRP (# 130-092-113)
- Anti-HA-HRP (# 130-091-972)

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- Anti-His-HRP (# 130-092-785)
- Anti-His-HRP (C-terminal) (# 130-092-783)
- Anti-GFP-HRP (# 130-091-833)
- Fluorochrome-conjugated Anti-Tag antibodies are also available: www.miltenyibiotec.com
- Multi-8 Columns, molecular (# 130-092-444)
- Multi-96 Columns, molecular (# 130-092-445)
- Deep Well Block (6 Deep Well Blocks, 2.5 mL, with adhesive sealing foil, # 130-092-549)
- MACS® Products for cell separation: www.miltenyibiotec.com
- MACSmolecular products and services for molecular analyses: www.miltenyibiotec.com

2. Protocol

Before starting

▲ Perform tissue or cell lysis on ice to prevent protein degradation. Protease inhibitors should be added to the lysis buffer. For details, see list with appropriate protease inhibitors and concentrations in section 5.1, Protease inhibitors.

▲ The lysis is the most crucial step during an immunoprecipitation. The Triton® X-100 lysis buffer is suitable for a wide range of protein sources, see section 1.4, Reagent and instrument requirements.

The Triton® X-100 lysis buffer can be replaced by a lysis buffer optimized to the experimental situation. The lysis buffer must not impair the antigen-antibody binding. Therefore, the lysis buffer conditions must be carefully chosen. Factors such as ionic strength, pH, concentration and type of detergent, presence of divalent cations, co-factors, and stabilizing ligands all influence the effectiveness of a lysis buffer. Generally, the lysis buffer should not contain SDS as it may disrupt the cell nuclei, see also Chapter 3, Tips & hints.

2.1 Sample preparation

▲ Pre-cool appropriate lysis buffer and centrifuge to 4 °C. Prepare ice bucket.

Lysis of adherent cells

1. Remove medium from culture dish.
2. Add 1 mL of pre-cooled (4 °C) lysis buffer to a 9 cm culture dish containing $1-10 \times 10^6$ cells. Scrape the cell lysate from the culture dish using a cell scraper and transfer to a 1.5 mL tube. Mix well and incubate for 30 minutes on ice with occasional mixing.
 - ▲ Note: Lysis of higher numbers of cells might lead to clogging of the columns and to lowered yields or purity of the eluate.
3. Centrifuge at $10,000 \times g$ for 10 minutes at 4 °C to sediment the cell debris.
4. Transfer the supernatant to a fresh 1.5 mL tube and proceed to section 2.2, Magnetic labeling and protein isolation.

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5. (Optional) The lysate can also be stored at this step at -20 °C or -70 °C. Lysate should be thawed on ice.

Lysis of suspension cells

1. Transfer the cells of one 9 cm culture dish containing $1-10 \times 10^6$ cells to a centrifugation tube and centrifuge at $300 \times g$ for 5 minutes at 4 °C.
2. Remove supernatant and place the tube containing the cell pellet on ice. Add 1 mL of pre-cooled (4 °C) lysis buffer and mix well.
 - ▲ Note: Lysis of higher numbers of cells might lead to clogging of the columns and to lowered yields or purity of the eluate.
3. Incubate on ice for 30 minutes with occasional mixing.
4. Centrifuge at $10,000 \times g$ for 10 minutes at 4 °C to sediment the cell debris.
5. Transfer the supernatant to a fresh 1.5 mL tube and proceed to section 2.2, Magnetic labeling and protein isolation.
6. (Optional) The lysate can also be stored at this step at -20 °C or at -70 °C. Lysate should be thawed on ice.

2.2 Magnetic labeling and protein isolation

▲ **Caution:** Read the MultiMACS Separator user manual carefully before running a process. Read the section Warnings and precautions, first chapter, before switching on the instrument. Always be sure that the MultiMACS 96 Magnet, the MultiMACS Column Holder, and the plates are in the same orientation, see MultiMACS Separator user manual for details.

▲ The MultiMACS Separator internal software provides various pre-defined separation programs, please visit www.miltenyibiotec.com for updates.

Pre-defined MultiMACS Separator programs

MULTI-8/96 POS is the standard process used for Multi-8 or Multi-96 Columns with the MultiMACS Epitope Tag Isolation Kits. In the final step the target protein is eluted from the MicroBeads.

If the flow-through should also be collected, the pre-defined process MULTI-8/96 NEG/POS should be used.

To run a process with different process parameters, a new program can be created or the parameters of an existing one can be edited. Please see details in the corresponding Chapter 6 of the MultiMACS Separator user manual.

Before starting

▲ For buffer preparation, see section 1.4, Reagent and instrument requirements.

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▲ For denaturing elution for SDS-PAGE pre-heat a heating block or PCR cycler to 95 °C. For rapid and convenient pipetting of the elution buffer, we recommend heating the buffer in tubes or in plates that are compatible with an 8- or 12-channel multipipette. To ensure a complete reduction of the target protein we recommend heating the eluate to 95 °C for 5 minutes following the elution step.

▲ Elution with other buffers or at temperatures below 95 °C is also possible.

▲ If using plates with other than standard height dimensions, use plates that comply to the ANSI/SBS standards and adjust the process parameter Plate Height, see MultiMACS Separator user manual.

2.2.1 Magnetic labeling

1. Add 50 µL of Anti-Tag MicroBeads to the lysate of 1–10×10⁶ cells to magnetically label the epitope-tagged proteins.
2. Mix well.
3. Incubate for 30 minutes on ice.

2.2.2 Multi8/96 Column preparation and loading

1. Switch on the MultiMACS 96 Separation Unit and touch the **Welcome Screen** or wait for a few seconds until the Process Selection Screen appears.

```

WELCOME TO THE
MULTIMACS SEPARATOR
BY MILTENYI BIOTEC
  
```

MULTI-8/96 POS	NEW	▲
MULTI-8/96 NEG/POS	SET UP	▼

▲ **Note:** The scroll function is only visible if there are more programs than displayed.

2. The last process performed on the MultiMACS Separator is displayed on the upper left of the screen (default: MULTI-8/96 POS). The second last process is listed below. If necessary, scroll through the list of available process names by touching the symbol ▲ or ▼ until MULTI-8/96 POS is displayed. Touch **MULTI-8/96 POS** to go to the Process Management Screen.

MULTI-8/96 POS	←	→
VIEW EDIT	ESC	▶

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3. If necessary, check the process parameters. See MultiMACS Separator user manual for details. Touch ▶ to start the process and to move the magnet to the start position. Follow the instructions given on the Touch Display.

INSERT MULTI-8/96 COLUMNS	MOVE BACK	OK
	ESC	

4. For purification of less than 96 samples

Unpack the necessary number of individual, sterile-packed Multi-8 Columns and put them in the MultiColumn Frame. Avoid touching the column tips to prevent protease contamination.

For purification of 96 samples

Unpack Multi-96 Columns. Avoid touching the column tips to prevent protease contamination.

5. Insert a MultiColumn Frame with up to twelve Multi-8 Columns or a pre-packed Multi-96 Column into the MultiMACS Column Holder. Touch **OK** to move the magnet upwards and for the next screen.

INSERT WASTE PLATE:	MOVE BACK	OK
DWB (44 MM)	ESC	

6. Place the waste plate, for example, Deep Well Block, 2.5 mL, onto the Tip-Touch Plate. If using a plate with a different height, adjust the process parameter Plate Height of the waste plate, for details see MultiMACS Separator user manual. Touch **OK** to move the MultiMACS 96 Magnet downwards. Column tips now slightly immerse in the waste plate. The following screen appears.

RINSE, APPLY SAMPLE, WASH. IF REQUIRED: PRE-ELUTE	MOVE BACK	OK
	ESC	

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- Prepare the Multi-8 columns by adding 200 μ L of Equilibration Buffer and let it pass through. Columns are “flow stop” and do not run dry.
- Place SDS-PAGE elution buffer in the pre-heated block. Pre-heat 90 μ L of buffer per separation column.
- After the labeling incubation has been completed, apply the cell lysate to the columns and let the lysate pass through. Magnetically labeled protein is retained in the Multi-8/96 Columns.

2.2.3 Wash

- Rinse the columns with 4 \times 200 μ L of wash buffer 1 (refer to section 1.4, Reagent and instrument requirements) or any other suitable buffer. Let the buffer pass through the columns.

▲ **Note:** In initial experiments the same buffer that was used for the lysis of the cells should be used for column washes. In order to reduce the background of non-specifically bound proteins, a more stringent wash buffer can be chosen for subsequent experiments, e.g. wash buffer 1 (refer to section 1.4 “Reagent and instrument requirements”). Co-immunoprecipitates are much more sensitive to stringent wash buffers; therefore, we recommend using only lysis buffer for all column washes.

- Rinse the columns with 100 μ L of wash buffer 2 (refer to section 1.4, Reagent and instrument requirements) and let the buffer pass through.

▲ **Note:** It is important that high concentrations of residual salt and detergent are removed from the immune complex prior to elution as both may interfere with a subsequent SDS-PAGE analysis.

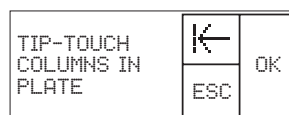
▲ **Note:** To perform enzymatic reactions on the column, proceed to section 5.3, In-column enzymatic reactions.

2.2.3 Elution with denaturing buffer

▲ For native elution, see section 5.2, Native protein elution.

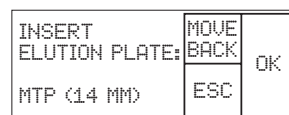
- Apply 20 μ L of pre-heated (95 $^{\circ}$ C) SDS-PAGE elution buffer (refer to section 1.4, Reagent and instrument requirements) directly onto the Multi-8 Column matrix using a fresh pipette tip for each column. Avoid contact with the Multi-8 Column reservoir. Incubate for 5 minutes at room temperature.

- Touch **OK** for the next screen.

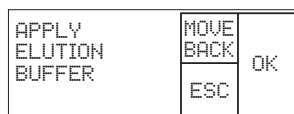


- Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the wells of the Deep Well Block touch the tips of the Multi-8 Columns. This process removes any drops on the column tips that did not fall off by gravity.

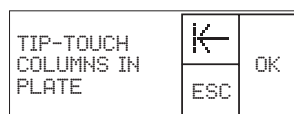
- Touch **OK** to move the MultiMACS 96 Magnet upwards.



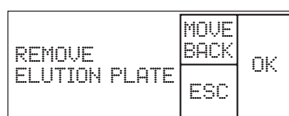
- Remove Deep Well Block. If some wells were unused, pour off waste liquid and store plate. Insert the elution plate (Microtiter Plate). If using a plate with a different height, adjust the process parameter Plate Height of the elution plate, see MultiMACS Separator user manual.
- Touch **OK** and the MultiMACS 96 Magnet will move downwards until column tips slightly immerse (1 mm) in the elution plate.



- Apply 50 μ L of pre-heated (95 $^{\circ}$ C) SDS-PAGE elution buffer directly onto the Multi-8 Column matrix using a fresh pipette tip for each column. Avoid contact with the Multi-8 Column reservoir.
- Wait approximately 1 minute until the elution buffer has passed through. Touch **OK** for the next screen.



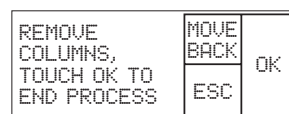
- Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the wells of the elution plate touch the tips of the Multi-8 Columns. This process removes any drops on the column tips that did not fall off by gravity. Touch **OK** to move the MultiMACS 96 Magnet upwards.



- Remove elution plate and use samples for downstream application. Alternatively, seal the plate with adhesive foil and store it at -20 $^{\circ}$ C or -70 $^{\circ}$ C.

▲ **Note:** To ensure complete reduction of the eluted target protein, incubating the eluate at 95 $^{\circ}$ C for an additional 5 minutes is recommended.

- Touch **OK** to move the MultiMACS 96 Magnet away from the Column Holder.



12. Remove Column Frame with Multi-8/96 Columns. If less than twelve Multi-8 Columns were used, remove the used Multi-8 Columns and store the Column Frame. Touch **OK** to finish the process.

3. Tips & hints

3.1 Lysis buffers

- **Standard lysis buffer:** the Triton X-100 lysis buffer (refer to section 1.4, Reagent and instrument requirement).

In case of co-immunoprecipitations with very weak protein-protein interactions, the use of low-salt lysis buffer (refer to section 1.4, Reagent and instrument requirement) is recommended.

If strong background ionic interactions are expected, the use of high-salt lysis buffer (refer to section 1.4, Reagent and instrument requirement) is recommended.

- **Bacterial cell lysis:** the Triton X-100 lysis buffer can be used to prepare a bacterial cell lysate by sonication.
- **Yeast cell lysis:** the Triton X-100 lysis buffer can be used to prepare a yeast cell lysate by glass bead disruption of the cell wall.

3.2 Buffers for mass spectrometry analysis

Mass spectrometry (MS) analysis is sensitive to high salt concentrations, detergents, and other buffer constituents. Thus, all components of the used buffers should be chosen carefully and buffer preparation should be optimized according to the characteristics of the isolated proteins or protein complexes and to the individual MS analysis.

As the provided Equilibration Buffer contains Triton X-100, which can interfere with MS measurements, it is recommended to use an alternative buffer and equilibrate the Multi-8/96 Columns by applying, for example, 200 μ L of 150 mM NaCl, 1% N-octyl glucopyranoside, 50 mM Tris HCl (pH 8.0). A detergent-containing buffer is essential for equilibration of the columns. For protein samples requiring detergents for solubilization, only N-octyl glucopyranoside is MS-compatible.

3.3 Function of buffer components

Detergents—these are partially hydrophobic and partially hydrophilic and can solubilize membranes and membrane proteins. They work to increase protein solubility and decrease aggregation. Non-ionic detergents tend to be more gentle in their actions than ionic detergents and are more suitable for protein-protein interaction studies.

Salts—increasing the salt concentration in the buffer will decrease ionic interactions between proteins in a cell lysate.

pH—increasing or decreasing the pH of the buffer will change the net charge of the proteins depending on their pI and therefore influence the extent of non-specific ionic interactions.

DTT—reducing agent that is often used to prevent loss of enzyme function via oxidation during protein isolation.

EDTA—this chelation agent binds divalent cations and can be used to prevent the action of certain enzymes that require ions such as Mg^{2+} or Ca^{2+} . It can also prevent protein-protein interactions that are dependent on the presence of cations. Please note that the Anti-Tag MicroBead storage buffer contains 5 mM EDTA.

Phosphatase inhibitors—when active kinases or phosphorylated proteins are to be isolated, we recommend the addition of 1 mM activated sodium orthovanadate (not compatible with DTT) and 1–10 mM NaF to inhibit phosphatase activity.

4. Troubleshooting

Slow column—if the buffer starts to run slowly through the columns it could either be due to cell debris occluding the column or to air bubble formation within the column. Cell debris should be efficiently removed by high-speed centrifugation of the lysate (> 10,000 \times g) before addition of the Anti-Tag MicroBeads. To prevent air bubble formation, use room-temperature buffers for the wash steps or—where possible—degas the buffers before use.

No protein recovery—may be caused by insufficient cell lysis. The lysis buffer should be altered to optimize the recovery of the tagged target

protein, please see section 3.1, Lysis buffers. Too stringent lysis or wash buffers may also impair the binding of antibody to the epitope tag; in this case, a lower salt concentration may help.

Protein background—if many background protein bands are present following SDS-PAGE analysis of the eluates, a more stringent lysis and/or wash buffer with higher salt concentrations should be used. For details, refer to section 3.1, Lysis buffers, or to section 3.3, The function of buffer components.

Background smear—if a smear is seen following SDS-PAGE analysis of the eluates, suitable protease inhibitors should be added to the lysis and wash buffers. For details see section 5.1, Protease inhibitors.

Variable elution volumes—reuse of pipette tips to pipette hot 1×SDS gel loading buffer often leads to the transfer of inexact volumes. Always use fresh tips to pipette hot gel loading buffer. Drops remaining on the column tip following the pre-elution or elution steps can result in higher or lower elution volumes. Carrying out a tip-touch step to remove drops from the column tips will ensure reproducible elution volumes.

5. Appendix

5.1 Protease inhibitors

Cocktails recommended, for example, PMSF, Leupeptin, Aprotinin:

S Serine proteases

M Metalloproteases

C Cysteine proteases

Inhibitor	Final concentration	Stock solution preparation
α1-Antitrypsin (S)	10 μM	6 mg/mL (1000×) in ddH ₂ O, pH 7
Aprotinin (S)	0.3 μM	1 mg/mL (500×) in ddH ₂ O, pH 7
Benzamidin (S)	2 mM	3 mg/mL (10×) in ddH ₂ O, pH 7
EDTA-Na ₂ (M)	1 mM	0.5 M (500×) in ddH ₂ O, pH 8
E-64 (C)	10 μM	0.36 mg/mL (100×) in 1:1 mixture ddH ₂ O, pH 7: EtOH
Leupeptin (S, C)	10 μM	5 mg/mL (1000×) in ddH ₂ O, pH 7
PMSF (S)	1 mM	17 mg/mL (100×) in Ethanol, Isopropanol or Methanol. Inactivated by DTT

5.2 Native protein elution

After washing the columns with wash buffer 2, section 2.2.3 step 2, a non-denaturing elution of the column-bound target protein is also possible: either by using a pH shift or by eluting the antigen–Anti-Tag MicroBead complex.

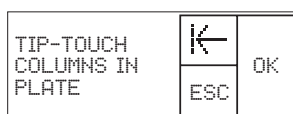
5.2.1 Elution by pH shift using triethylamine (pH 11.8)

Before starting

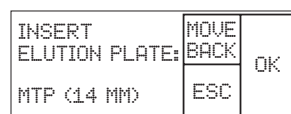
Prepare the elution plate (Microtiter Plate) by pipetting 3 μL of 1 M MES (pH 3) per well for neutralization.

Proceed after section 2.2.3, step 2.

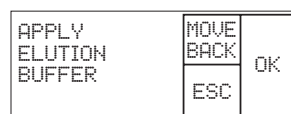
- Rinse columns additionally with 100 μL of wash buffer 2.
- Apply 20 μL of 0.1 M triethylamine (pH 11.8), 0.1% Triton X-100 directly onto the Multi-8 Column matrix using a fresh pipette tip for each column. Avoid contact with the Multi-8 Column reservoir. Incubate for 5 minutes at room temperature.
- Touch **OK** for the next screen.



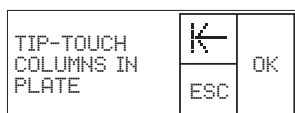
- Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the wells of the Deep Well Block touch the tips of the Multi-8 Columns. This process removes any drops on the column tips that did not fall off by gravity.
- Touch **OK** to move the MultiMACS 96 Magnet upwards.



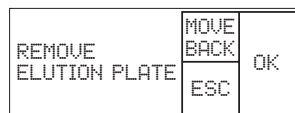
- Remove Deep Well Block. If some wells were unused, pour off waste liquid and store plate.
- Insert elution plate containing 3 μL of 1 M MES (pH 3) per well for neutralization. If using a plate with a different height, adjust the process parameter Plate Height of the elution plate, see MultiMACS Separator user manual.
- Touch **OK** and the MultiMACS 96 Magnet will move downwards until column tips slightly immerse (1 mm) in the elution plate.



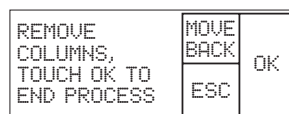
9. Apply 50 μL of 0.1 M triethylamine (pH 11.8) 0.1% Triton X-100 directly onto the Multi-8 Column matrix using a fresh pipette tip for each column. Avoid contact with the Multi-8 Column reservoir.
10. Wait approximately 1 minute until the elution buffer has passed through.
11. (Optional) For higher recovery we recommend a second elution step. Apply 50 μL of 0.1 M triethylamine (pH 11.8) 0.1% Triton X-100 onto the Multi-8 Column matrix. Wait approximately 1 minute until the elution buffer has passed through.
12. Touch **OK** for the next screen.



13. Move the Tip-Touch Plate firmly back and forth once so that the inner walls of the wells of the elution plate touch the tips of the Multi-8 Columns. This process removes any drops on the column tips that did not fall off by gravity. Touch **OK** to move the MultiMACS 96 Magnet upwards.



14. Remove elution plate and use samples for downstream application. Alternatively, seal the plate with adhesive foil and store it at $-20\text{ }^{\circ}\text{C}$ or $-70\text{ }^{\circ}\text{C}$.
15. Touch **OK** to move the MultiMACS 96 Magnet away from the Column Holder.



16. Remove Column Frame with Multi-8/96 Columns. If less than twelve Multi-8 Columns were used, remove the used Multi-8 Columns and store the Column Frame. Touch **OK** to finish the process.

5.3 In-column enzymatic reactions

In-column enzymatic reactions with the immunoprecipitated epitope-tagged protein complex can be carried out while the protein remains bound to the Multi-8/96 Columns. Performing the reaction on the column offers the advantage of very convenient handling, especially when working with radioactively labeled proteins or substrates. It also allows a serial enzymatic reaction to be performed on the same column.

A few guidelines are listed below on how to perform the enzymatic reaction in the column; however, every enzymatic reaction should be performed with individually optimized conditions.

▲ The lysis of the cells and the magnetic labeling should be performed as described in section 2.2 and 2.3. However, neither lysis buffer nor wash buffer should contain SDS since it may impair the biological activity of the immunoprecipitated complex. The wash buffer and number of wash steps should be optimized for efficient removal of non-specific proteins. Prior to the enzymatic reaction, the column should be rinsed with 100 μL of reaction buffer used for the enzymatic reaction.

▲ The void volume of the Multi-8/96 Columns is 30 μL . Thus, buffers and solutions used for incubation with the immobilized immunoprecipitate should always be applied in 30 μL aliquots. If incubations with volumes higher than 30 μL is necessary, sequentially incubate in 30 μL aliquots until the total volume has been applied.

▲ Following enzymatic reaction, the immunoprecipitated protein (complexes) can be eluted for SDS-PAGE analysis.

5.3.1 Protocol for in-column enzymatic reaction

Cell lysis and magnetic labeling should be performed as described in section 2.2 and 2.3. Proceed after completing wash steps, section 2.2.3, step 2.

▲ **Note:** Neither lysis nor wash buffer should contain SDS since it may impair the biological activity of the immunoprecipitated complex. Choose type of wash buffer and number of wash steps to remove non-specific protein.

Before starting

▲ Prepare sufficient amounts of 1 \times reaction buffer and wash buffer 2 (each 100 μL /reaction).

▲ To collect both the flow-through and column-bound fractions, the predefined process MULTI-8/96 NEG/POS should be used.

1. Rinse the columns with 100 μL of wash buffer 2 and let the buffer pass through.
2. Rinse the Multi-8 Column with 1 \times 100 μL of enzymatic reaction buffer.
3. (a) If the target to be analyzed is **in the flow-through**, carry out a tip-touch and remove the Deep Well Block. Insert the elution plate (Microtiter Plate) and press **OK**.
(b) If the target to be analyzed is **bound to the columns**, continue with step 4.
4. Apply enzyme solution to the Multi-8/96 Columns in 30 μL aliquots. After the enzymatic reaction has been performed, either the non-

bound fraction (pooled flow-through) can be analyzed, or the immunoprecipitated protein can be eluted for further analysis.

Wash

1. Rinse the Multi-8/96 Column with 1×100 µL of reaction buffer to remove remaining enzyme or substrate for analysis.
2. Proceed with section 2.2.3, Wash, step 2: Rinse the columns with 100 µL wash buffer 2 and let the buffer pass through.

Elution

1. Proceed with section 2.2.4, Elution with denaturing buffer or section 5.2, Native protein elution.

6. References

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5. Wilson, I. A. *et al.* (1984) The structure of an antigenic determinant in a protein. *Cell* 37: 767–778.
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9. Smith, D. B. and Johnson, K. S. (1988) Single-step purification of polypeptides expressed in *Escherichia coli* as fusions with glutathione-S-transferase. *Gene* 67: 31–40.

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