µMACS™ Streptavidin Kit

Order No. 130-074-101

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1. Description

Components	**2 mL µMACS™ Streptavidin MicroBeads**
	4 mL Equilibration Buffer for protein applications (for column equilibration)
	4 mL Equilibration Buffer for nucleic acid applications (for column equilibration)
	20 µ Columns (µ Columns cannot be used for cell separation)
Size	For up to 20 reactions.
Product format	µMACS Streptavidin MicroBeads are supplied as a suspension containing stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 4 – 8 °C. Do not freeze. The expiration date is indicated on the vial label. Store µ Columns at room temperature, dry and protected from light.

1.1 Background and product applications

The µMACS Streptavidin Kit with µMACS Streptavidin MicroBeads has been developed for the magnetic labeling and isolation of biotinylated molecules and their interaction partners. The µMACS MicroBeads contained in this kit are a colloidal suspension of extremly small (50 nm in diameter) super-paramagnetic MicroBeads that are conjugated to streptavidin, which enables a fast and effective binding to biotinylated molecules. Thereby a biotinylated probe, e.g. an oligonucleotide, DNA, RNA, or protein can be used to specifically isolate a target molecule, e.g. a hybridizing DNA fragment or a DNA-binding protein. By magnetic labeling with µMACS Streptavidin MicroBeads, this molecular complex is retained in a µ Column placed in the magnetic field of a µMACS or thermoMACS Separator. Stringent washing steps can be easily applied to remove non-specifically binding molecules. Afterwards, the non-biotinylated target molecules can be eluted with high purity, whereas the magnetically-labeled biotinylated probe remains bound to the column. For an overview and a general working scheme see figure 1.

Examples of applications

- Isolation of specific DNA and RNA molecules, e.g. viral DNA or tRNA, with complementary biotinylated nucleic acid probes.
- Direct isolation of biotinylated molecules (bound to µMACS MicroBeads).
- Isolation of DNA- and RNA-binding proteins with biotinylated nucleic acid probes.
- Protein interaction studies, e.g. isolation of receptors by using biotinylated ligands.
- Isolation of complexes, organelles, or viruses.
- Serial reactions with the immobilized molecules in the column (e.g. in-column enzymatic modifications of biotinylated molecules).

1.2 Reagent and instrument requirements

- µMACS or thermoMACS Separator and MultiStand.
- Appropriate buffers, e.g. for cell lysis, washing steps, and for elution of the target molecule (e.g. high-salt or low pH elution buffer).

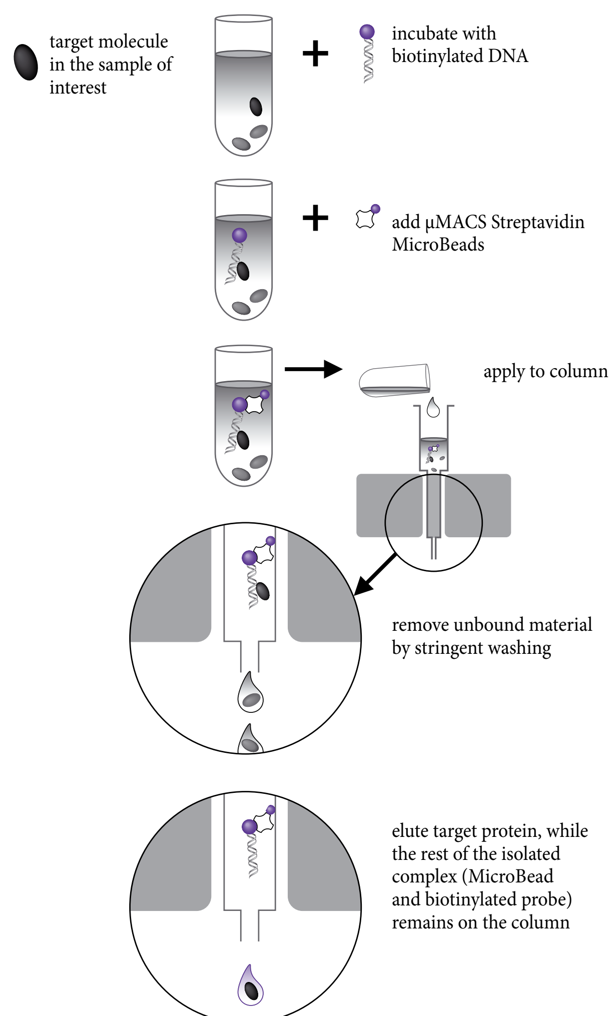

Figure 1: Isolation of target molecules with biotinylated probes: here the isolation of DNA-binding proteins with biotinylated DNA fragments is shown.

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2. General Protocol

▲ The described protocol is a general handling recommendation for the µMACS Streptavidin MicroBeads which works for a variety of applications. For more specific applications special protocols are available on the website www.miltenyibiotec.com/protocols or from the technical service of the local supplier.

2.1 Magnetic labeling

Biotinylated molecules (e.g. biotinylated oligonucleotides) can be purchased from a variety of different suppliers. Alternatively, kits for the biotinylation of nucleic acids or proteins are commercially available.

For biotinylation of DNA, we recommend using 5'biotinylated HPLC-purified primers in a PCR reaction. Alternatively, biotinylated UTP/dUTP or other NTPs/dNTPs can be used to modify nucleic acids by in-vitro transcription (RNA), PCR, nick translation, 3'end labeling or else.

▲ Free biotin should be removed before labeling of target molecules.

1. Incubate biotinylated molecules with the sample (e.g. a cell lysate) containing the target molecules.

   ▲ **Note:** Make sure there are no clumps in the cell lysate/binding reaction which may lead to clogging of the column.

2. After complex formation add an appropriate amount of µMACS Streptavidin MicroBeads and mix.

   ▲ **Note:** 100 µL of the µMACS Streptavidin MicroBeads bind up to 100 pmol biotinylated molecules (see Appendix). Binding is completed in seconds, prolonged incubation is not necessary.

2.2 Magnetic isolation

▲ Use appropriate equilibration buffer (supplied with the kit) for protein or nucleic acid applications. If the application includes proteins and nucleic acids, using the equilibration buffer for protein applications is recommended, as the equilibration buffer for nucleic acids contains nucleic acid-protecting agents which may affect your protein.

▲ Generally the buffer used for the binding reaction can also be used for the washing steps. If more stringent washing steps are necessary to remove non-specifically bound molecules, the salt concentration in the wash buffer should be increased (protein applications) or decreased (nucleic acid applications). Increasing the temperature of the washing buffer will also decrease binding of non-specific molecules.

1. Place a µ Column in the magnetic field of the µMACS separator. Prepare column by rinsing with 1×100 µL of the appropriate equilibration buffer.

2. Rinse the column with 2×100 µL of the same buffer as used for the binding reaction between your biotinylated molecules and the target molecules.

3. Apply the binding reaction onto the top of the column matrix.

4. Rinse the column with at least 4×100 µL of an appropriate buffer to remove non-specifically binding molecules.

5a. Elution of target molecules bound to a biotinylated probe: Apply 150 µL of an appropriate elution buffer directly onto the top of the column matrix.

   ▲ **Note:** In most applications, the second to fourth drop will contain the target molecules. If it is not possible to check the content of the drops, collect the total elution volume.

5b. Elution of biotinylated molecules: Remove the column out of the magnetic field. Apply 150 µL of an appropriate elution buffer (e.g. TE buffer for DNA) directly onto the top of the column.

   ▲ **Note:** The eluate contains the biotinylated molecule bound to µMACS Streptavidin MicroBeads.

3. Appendix: How to estimate the binding capacity of µMACS™ Streptavidin MicroBeads for particular biotinylated nucleic acids or proteins

100 µL µMACS™ Streptavidin MicroBeads bind up to 100 pmol biotinylated molecules.

100 µL µMACS Streptavidin MicroBeads bind up to X µg of DNA/RNA.
X is calculated as followed:
X =length of DNA/RNA(basepairs)×0.066 (for ds-nucleic acids with one biotin)
X =length of DNA/RNA(basepairs)×0.033 (for ds-nucleic acids with two biotins)
X =length of DNA/RNA (bases)×0.033 (for ss-nucleic acids with one biotin)
X =length of DNA/RNA (bases)× 0.017 (for ss-nucleic acids with two biotins)

100 µL µMACS Streptavidin MicroBeads bind up to X µg protein with an average of Y biotins.
X is calculated as follows:
X = mol. weight (kDa) / (10×Y)
Y is the average number of biotin molecules per protein molecule.

Examples

A. 800 bp PCR fragment with 1 biotin. How many µg PCR fragment is maximally bound by 100 µL µMACS Streptavidin MicroBeads?
X µg = 800×0.066 = 53 µg
why? 1 bp => 660 g/mol => 660 pg/pmol
800 bp => 800×660 pg/pmol => 528000 pg/pmol
ergo: 1 pmol PCR fragment => 1 pmol biotin => 528000 pg
for 100 µL µMACS Streptavidin MicroBeads:
up to 100 pmol biotin => 528000×100 pg = 52800000 pg = 52.8 µg

B. 50 kDa Protein with 4 biotins (average). How many µg protein is maximally bound by 100 µL µMACS Streptavidin MicroBeads?
X µg = 50 / (10×4) = 1.25 µg
why? 50 kDa => 50000 g/mol => 50000 pg/pmol
ergo: 1 pmol protein => 4 pmol biotin => 50000 pg
that means: 1 pmol biotin => 12500 pg
for 100 µL µMACS Streptavidin MicroBeads:
up to 100 pmol biotin => 12500×100 pg = 1250000 pg = 1.25 µg

▲ Steric properties of large molecules may cause a lower binding capacity.

Warnings

Reagents contain sodium azide. Under acidic conditions sodium azide yields hydrazoic acid, which is extremely toxic. Azide compounds should be diluted with running water before discarding. These precautions are recommended to avoid deposits in plumbing where explosive conditions may develop.

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