# µMACS™ Streptavidin Kit

Order no. 130-074-101

## Content



## 1. Description

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| **Components** | **2 mL µMACS™ Streptavidin MicroBeads**<br>**4 mL Equilibration Buffer for protein applications**<br>**4 mL Equilibration Buffer for nucleic acid applications**<br>**20 µ Columns** |
| **Capacity** | For up to 20 reactions. |
| **Product format** | µMACS Streptavidin MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide. |
| **Storage** | Store µ Columns at room temperature, dry, and protected from light.<br>Store all other components protected from light at 2–8 °C. Do not freeze. The expiration date is indicated on the vial label. |

### 1.1 Background information

The µMACS Streptavidin Kit has been developed for the magnetic labeling and isolation of biotinylated molecules and their interaction partners. The µMACS Streptavidin MicroBeads contained in this kit are a colloidal suspension of extremly small (50 nm in diameter) super-paramagnetic MicroBeads conjugated to streptavidin, which enable a fast and effective binding to biotinylated molecules. These biotinylated molecules such as oligonucleotides, DNA, RNA, or proteins can be used as probes to specifically isolate a target molecules by magnetic labeling with µMACS Streptavidin MicroBeads. The labeled 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 refer to figure 1.

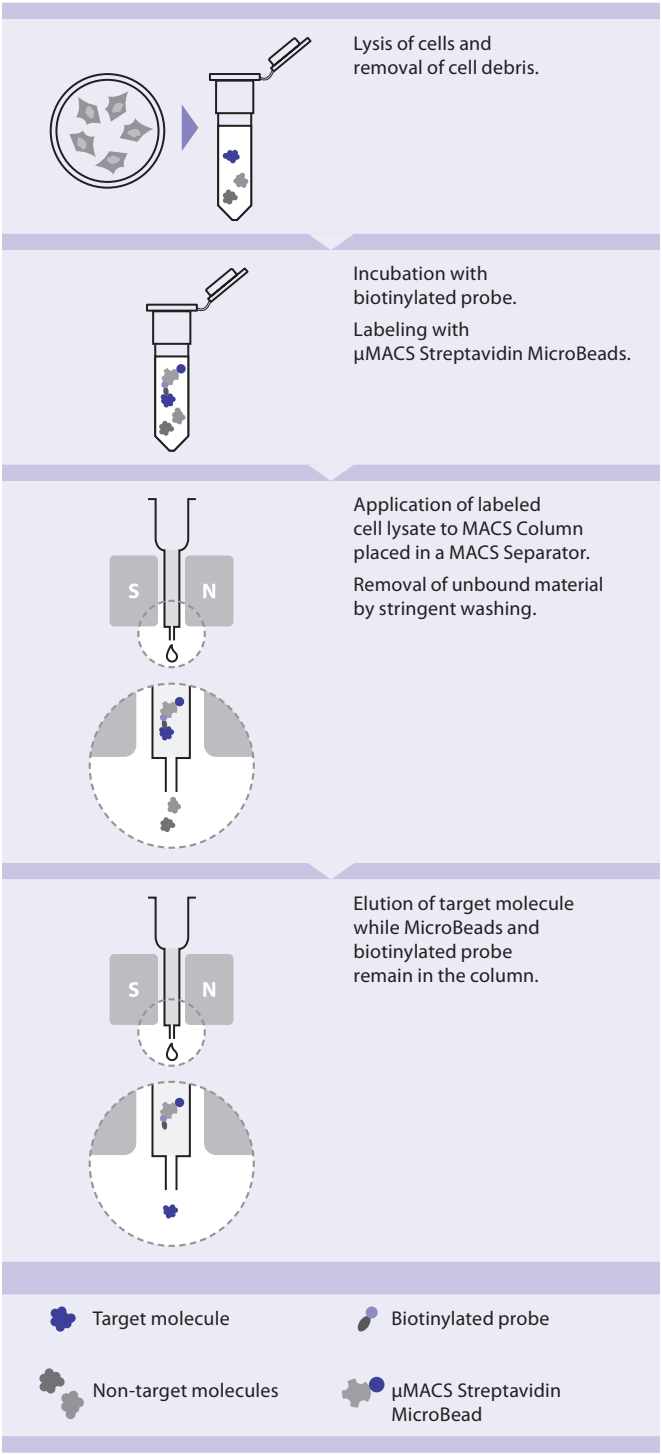


**Figure 1:** General workflow for isolation of target molecules with biotinylated probes using µMACS Streptavidin MicroBeads.


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### 1.2 Applications

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

### 1.3 Reagent and instrument requirements

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

## 2. Protocol

▲ The described protocol is a general recommendation for the µMACS Streptavidin MicroBeads which works for a variety of applications. For more specific applications special protocols are available on www.miltenyibiotec.com/protocols.

### 2.1 Magnetic labeling

For biotinylation of DNA, it is recommended to use 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, PCR, nick translation, 3'-end labeling, or other procedures.

▲ 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. The incubation time and temperature depends on the type of molecular interactions being investigated.

   ▲ **Note:** Make sure there are no clumps in the sample 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 (refer to 3. Appendix). Binding is completed in seconds, prolonged incubation is not necessary.

### 2.2 Magnetic isolation

▲ Use appropriate Equilibration Buffer for protein or nucleic acid applications. If the application includes both 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 the 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 a µMACS Separator or a thermoMACS 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 magnetically labeled complex onto the top of the column matrix.
4. Rinse the column with at least 4×100 µL of washing 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 from 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 => 528,000 pg/pmol
ergo: 1 pmol PCR fragment => 1 pmol biotin => 528,000 pg
For 100 µL µMACS Streptavidin MicroBeads:
up to 100 pmol biotin => 528,000×100 pg = 52,800,000 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 => 50,000 g/mol => 50,000 pg/pmol
ergo: 1 pmol protein => 4 pmol biotin => 50,000 pg
that means: 1 pmol biotin => 12,500 pg
For 100 µL µMACS Streptavidin MicroBeads:
up to 100 pmol biotin => 12,500×100 pg = 1,250,000 pg = 1.25 µg

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

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com for local Miltenyi Biotec Technical Support contact information.

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