Miltenyi Biotec

MACSflex™ MicroBead Kit, 0.5 mg

Order no. 130-105-806

Contents

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.

1. Description

This product is for research use only.

Components	**1 vial MACSflex MicroBeads, 0.5 mg – lyophilized** **2 mL MACSflex Reconstitution Buffer** **1 mL MACSflex Equilibration Buffer (for column equilibration)** **1.4 mL MACSflex Stop Reagent** **10 mL MACSflex Storage Buffer**
Capacity	For up to 500 pmol biomolecule.
Product format	Lyophilized MACSflex MicroBeads. MACSflex Storage Buffer contains stabilizer and 0.05% sodium azide.
Storage	Store protected from light at 2–8 °C. Do not freeze. For information about reconstitution of the lyophilized MicroBeads refer to chapter 2.

1.1 Principle of the MACSflex MicroBead Kit

The MACSflex MicroBeads are optimized for rapid, straight forward coupling to biomolecules and provided in a lyophilized format for easy reconstitution. After incubation with the biomolecule of choice, MACSflex MicroBeads are purified over µ Columns for immediate use. The ligand must carry an amino group and is coupled to the pre-activated MACSflex MicroBeads via N-hydroxy-succinimide (NHS). The recommended amount of biomolecule is within the range of 50 to 1000 pmol per milligram of MACSflex MicroBeads. If coupling and subsequent downstream use of the conjugated MACSflex MicroBeads are planned for the same day, the coupling can be stopped using the MACSflex Stop Reagent followed by column purification of the MACSflex MicroBeads. If storage of the coupled MACSflex MicroBeads is intended for later use, the coupling reaction should be performed overnight followed by column purification.

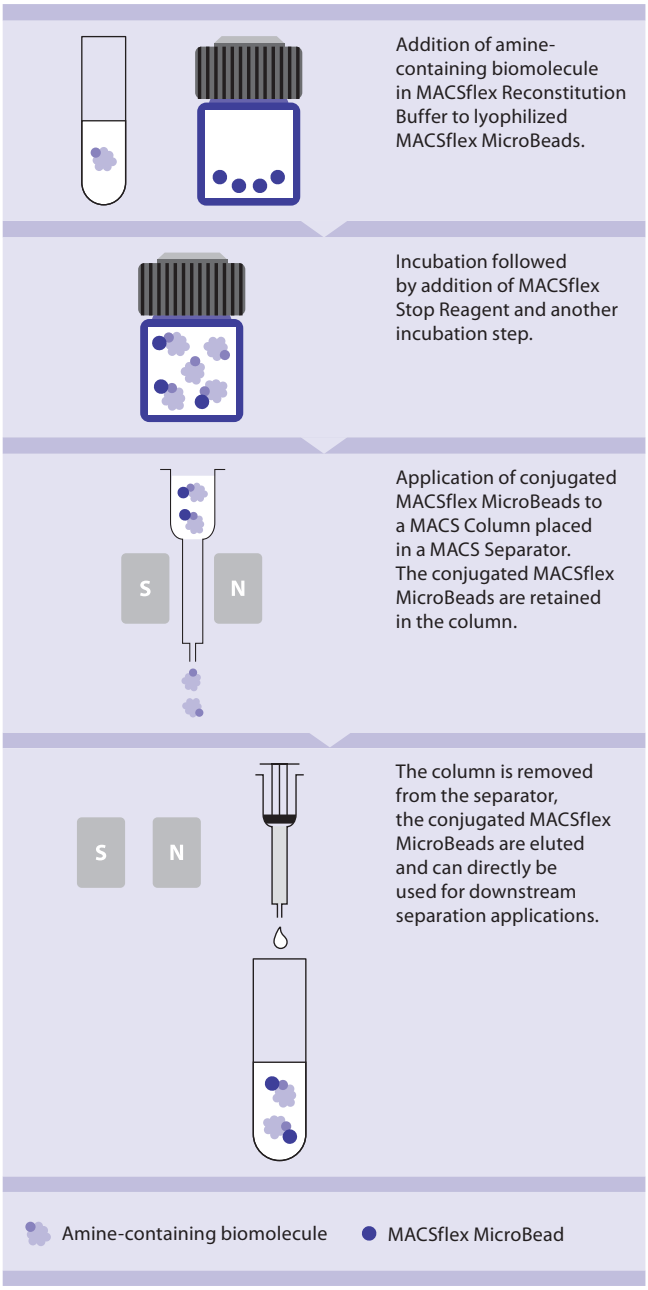

Figure 1: Overview

Miltenyi Biotec B.V. & Co. KG
Friedrich-Ebert-Straße 68, 51429 Bergisch Gladbach, Germany
Phone +49 2204 8306-0, Fax +49 2204 85197
macsde@miltenyi.com
www.miltenyibiotec.com

140-004-623.04

1.2 Background information

The MACSflex MicroBead Kits were developed for covalent coupling of biomolecules for use in molecular applications using MACS® Technology. Once coupled to the biomolecules, MACSflex MicroBeads can be used directly in downstream applications such as epitope tagged protein isolations and organelles isolations. Suitable starting protocols can be found on www.miltenyibiotec.com/130-105-806. If the coupled MACSflex MicroBeads are not intended to be used immediately, they can be coupled overnight followed by a washing step, for storage up to 4 weeks at 2–8 °C. The MACSflex MicroBeads exhibit all advantages of µMACS™ MicroBead technology including very low non-specific binding, easy handling, minimal hands-on time, and high sensitivity due to the small size of the MicroBeads. Flexible coupling to nearly any primary amine-containing biomolecule makes MACSflex MicroBeads an ideal choice for many downstream applications.

1.3 Applications

- Immunoprecipitation and co-immunoprecipitation of proteins and protein complexes
- Isolation of organelles like exosomes
- Isolation of specific DNAs or RNAs
- Isolation of specific DNA- or RNA-binding molecules
- Isolation of viruses

1.4 Reagent and instrument requirements

- µMACS Separator (# 130-042-602) or thermoMACS™ Separator (# 130-091-136) and MACS MultiStand (# 130-042-303)
- µ Columns (# 130-042-701)
- Amine-containing biomolecule in a maximum of 50 µL appropriate buffer

  ▲ **Note:** The buffer must not contain primary amines like Tris or glycine.

2. General protocol for coupling of biomolecules to MACSflex MicroBeads

1. Mix 450 µL of MACSflex Reconstitution Buffer and up to 50 µL of amine-containing biomolecule (provided in an appropriate buffer like 50 mM MES pH 6.0) using an appropriate reaction tube.

   ▲ **Note:** If the biomolecule amount exceeds the volume of 50 µL it has to be concentrated prior to mixing, e.g., by using a spin concentrator.

   ▲ **Note:** Primary amine-containing buffers (e.g. Tris or glycine) inhibit coupling of the ligand to MACSflex MicroBeads. Remove primary amine-containing buffer by buffer exchange methods like size-exclusion chromatography, dialysis, or desalting. For best results the buffer should be exchanged into 50 mM MES, pH 6.0.

2. Transfer the biomolecule-mixture completely to a glass vial containing the lyophilized MACSflex MicroBeads and reconstitute by pipetting up and down until resuspended.

3. Incubate the MACSflex MicroBeads for 2 hours at room temperature (18–25 °C).

   ▲ **Note:** If the ligand-coupled MACSflex MicroBeads are used for downstream applications the following day or later, please proceed with step 5B instead of step 3. The ligand-coupled MACSflex MicroBeads can be stored up to 4 weeks at 2–8 °C protected from light after column purification (steps 6–10).

4. (Optional) Add 37.5 µL of MACSflex Stop Reagent and vortex.

   ▲ **Note:** If the ligand-couple MACSflex MicroBeads are used for downstream applications within the same working day, stopping the reaction by addition of MACSflex Stop Reagent is mandatory.

5. A: If MACSflex Stop Reagent was added: Incubate for 20 minutes at room temperature (18–25 °C).

   B: If no MACSflex Stop Reagent was added: Incubate overnight at 2–8 °C protected from light.

6. Place a µ Column in the magnetic field of the µMACS Separator. Prepare column by rinsing with 1×200 µL of MACSflex Equilibration Buffer.

7. Apply the sample onto the top of the column matrix.

8. Wash the column with 3×200 µL of MACSflex Storage Buffer to remove unbound ligand.

9. Remove µ Column from the separator and place it on a suitable collection tube.

10. Elute the MACSflex MicroBeads with 2×125 µL MACSflex Storage Buffer. Collect the eluate in one single tube.

11. (Optional) If MACSflex MicroBeads were coupled overnight (step 5B) they can be stored for up to 4 weeks at 2–8 °C protected from light in MACSflex Storage Buffer. Otherwise they have to be used directly for downstream applications.

3. Reference

1. Hermanson, G. T. (2013) Bioconjugate Techniques, 3rd Edition, Elsevier Inc., USA: 233–234.

4. Troubleshooting

Clear eluate – if the two hours incubation was performed (step 3) but the reaction was not stopped using MACSflex Stop Reagent, the yield of MACSflex MicroBeads might be reduced and the solution appears clear instead of brownish. For the overnight incubation MACSflex Stop Reagent does not have to be used.

Ligand did not couple – can be caused by using amine-containing buffer. The buffer has to be free of amines and the pH of the buffer containing the ligand should not be >7.5.

Reconstitution without ligand – if the ligand was not added prior to the reconstitution of the MACSflex MicroBeads, it can be added subsequently. The ligand can be added up to 1 hour after reconstitution but this might lead to loss of performance.

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.

140-004-623.03

Legal notices

Limited product warranty

Miltenyi Biotec B.V. & Co. KG and/or its affiliate(s) warrant this product to be free from material defects in workmanship and materials and to conform substantially with Miltenyi Biotec's published specifications for the product at the time of order, under normal use and conditions in accordance with its applicable documentation, for a period beginning on the date of delivery of the product by Miltenyi Biotec or its authorized distributor and ending on the expiration date of the product's applicable shelf life stated on the product label, packaging or documentation (as applicable) or, in the absence thereof, ONE (1) YEAR from date of delivery ("Product Warranty"). Miltenyi Biotec's Product Warranty is provided subject to the warranty terms as set forth in Miltenyi Biotec's General Terms and Conditions for the Sale of Products and Services available on Miltenyi Biotec's website at www.miltenyibiotec.com, as in effect at the time of order ("Product Warranty"). Additional terms may apply. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING IF A PRODUCT IS SUITABLE FOR CUSTOMER'S PARTICULAR PURPOSE AND APPLICATION METHODS.

Technical information

The technical information, data, protocols, and other statements provided by Miltenyi Biotec in this document are based on information, tests, or experience which Miltenyi Biotec believes to be reliable, but the accuracy or completeness of such information is not guaranteed. Such technical information and data are intended for persons with knowledge and technical skills sufficient to assess and apply their own informed judgment to the information. Miltenyi Biotec shall not be liable for any technical or editorial errors or omissions contained herein.

All information and specifications are subject to change without prior notice. Please contact Miltenyi Biotec Technical Support or visit www.miltenyibiotec.com for the most up-to-date information on Miltenyi Biotec products.

Licenses

This product and/or its use may be covered by one or more pending or issued patents and/or may have certain limitations. Certain uses may be excluded by separate terms and conditions. Please contact your local Miltenyi Biotec representative or visit Miltenyi Biotec's website at www.miltenyibiotec.com for more information.

The purchase of this product conveys to the customer the non-transferable right to use the purchased amount of the product in research conducted by the customer (whether the customer is an academic or for-profit entity). This product may not be further sold. Additional terms and conditions (including the terms of a Limited Use Label License) may apply.

CUSTOMER'S USE OF THIS PRODUCT MAY REQUIRE ADDITIONAL LICENSES DEPENDING ON THE SPECIFIC APPLICATION. THE CUSTOMER IS SOLELY RESPONSIBLE FOR DETERMINING FOR ITSELF WHETHER IT HAS ALL APPROPRIATE LICENSES IN PLACE. Miltenyi Biotec provides no warranty that customer's use of this product does not and will not infringe intellectual property rights owned by a third party. BY USE OF THIS PRODUCT, THE CUSTOMER AGREES TO BE BOUND BY THESE TERMS.

Trademarks

MACS, MACSflex, the Miltenyi Biotec logo, thermoMACS, and µMACS are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide.