Miltenyi Biotec

MACSflex™ MicroBead Kit, 2 mg

Order no. 130-105-805

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, 2 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 2 nmol 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 MACSflex 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 or M 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.

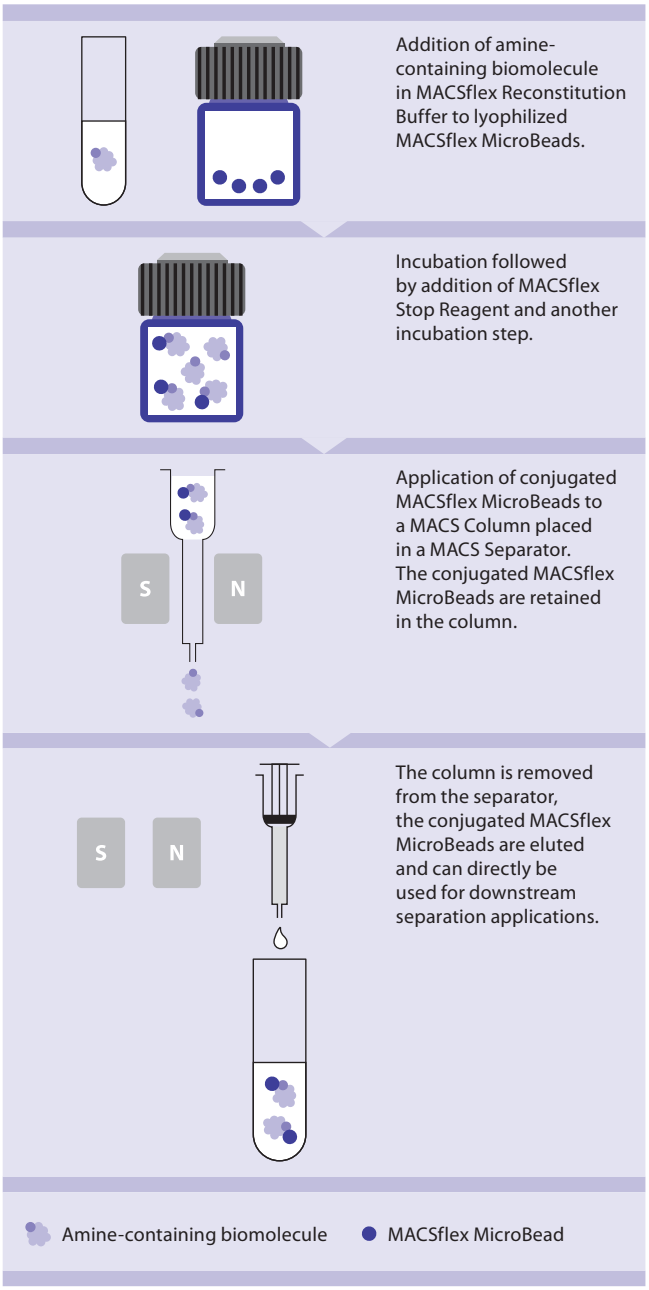

Figure 1: Overview.

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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/130-105-805. 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

- µ Columns (# 130-042-701), µMACS Separator (# 130-042-602) or thermoMACS™ Separator (# 130-091-136), and MACS MultiStand (# 130-042-303).
  Alternatively, M Columns (# 130-042-801) in combination with the MiniMACS™ Separator (# 130-042-102) and MACS MultiStand (# 130-042-303).
- Amine-containing biomolecule in a maximum of 200 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

2.1 Using µ Columns

1. Mix 1.8 mL of MACSflex Reconstitution Buffer and up to 200 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 200 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 150 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 4 µ Columns in the magnetic fields of the µMACS Separator. Prepare columns by rinsing each column with 1×200 µL of MACSflex Equilibration Buffer.

7. Apply 500 µL of the reaction onto the top of the column matrix of each column.

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

9. Remove columns from the separator and place them on suitable collection tubes.

10. Elute the MACSflex MicroBeads with 2×125 µL MACSflex Storage Buffer per column. Collect the eluate of each column in one single tube. Afterwards the eluted fractions can be pooled.

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.

2.2 Using M Columns

1. Mix 1.8 mL of MACSflex Reconstitution Buffer and up to 200 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 200 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 150 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.

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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 M Column in the magnetic field of the MiniMACS Separator. Prepare column by rinsing with 1×500 µL of MACSflex Equilibration Buffer.

7. Apply the complete reaction onto the top of the column matrix.

8. Wash the column with 3×400 µL of MACSflex Storage Buffer to remove unbound ligand.

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

10. Elute the MACSflex MicroBeads with 2×500 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.

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