Miltenyi Biotec

MACSprep™ Forensic Sperm MicroBead Kit
human

Order no. 130-125-280

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 mL Anti-Spermatozoa MicroBeads, human:** MicroBeads conjugated to monoclonal anti-human spermatozoa antibodies (isotype: recombinant human IgG1).
	4 mL Free-DNA Removal Buffer (10×)
	3×50 mL MACSprep™ Forensic Buffer
	1 vial Enzyme A - lyophilized
	1 mL Buffer A
Capacity	For 25 tests.
Product format	Anti-Spermatozoa MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.
Storage	Upon arrival immediately store all components at 2–8 °C. Do not freeze. The expiration date is indicated on the vial labels. For information about reconstitution and storage after reconstitution of the lyophilized component refer to chapter 2.2.

1.1 Principle of the MACS® Separation

The MACSprep™ Forensic Sperm MicroBead Kit, human has been developed for the fast isolation of sperm cells from standard proteinase K treated samples without performing differential lysis. After proteinase K treatment, the free DNA is removed and the isolation of sperm cells is performed with magnetic separation. Sperm cells are magnetically labeled with Anti-Spermatozoa MicroBeads, which recognizes a specific antigen on mature sperm head. Then, the cell suspension is loaded onto a MACS® Column, which is placed in the magnetic field of a MACS Separator. The magnetically labeled sperm cells are retained within the column. The unlabeled non-sperm cells run through; this cell fraction contains epithelial cells. After removing the column from the magnetic field, the magnetically retained sperm cells can be eluted as the positively selected cell fraction

1.2 Background information

The MACSprep Forensic Sperm MicroBead Kit, human has been developed for the positive selection of human sperms from cell mixtures. It contains an enzyme and buffers to remove the majority of non-spermatic or free DNA after recovering cells from swab samples. The human sperms, which acquired epididymal secreted proteins during maturation ,are then magnetically labeled with Anti-Spermatozoa MicroBeads and separated from the cell mixture.

1.3 Applications

- The purified sperm cells from forensic samples are well suited for further molecular analysis such as PCR, short tandem repeat (STR) , or sequencing analysis.

1.4 Reagent and instrument requirements

- MS Columns (# 130-042-201)
- MiniMACS™ Separator (# 130-042-102) or OctoMACS™ Separator (# 130-042-109)
- MACS MultiStand (# 130-042-303)
- OctoMACS Acrylic Tube Rack (# 130-090-448)
- Proteinase K, recombinant, PCR Grade, (e.g., Roche, 3115844001), working dilution is 100 µg/mL in MACSprep Forensic Buffer
- Nuclease-free water or double-distilled H_2O (ddH_2O)
- 1.5 mL low-binding microcentrifuge tubes
- DNA IQ™ Spin Baskets (e.g., Promega, # V1225)
- Microcentrifuge, pre-cooled to 4 °C
- Thermomixer suitable for 1.5 mL tube at 25 °C

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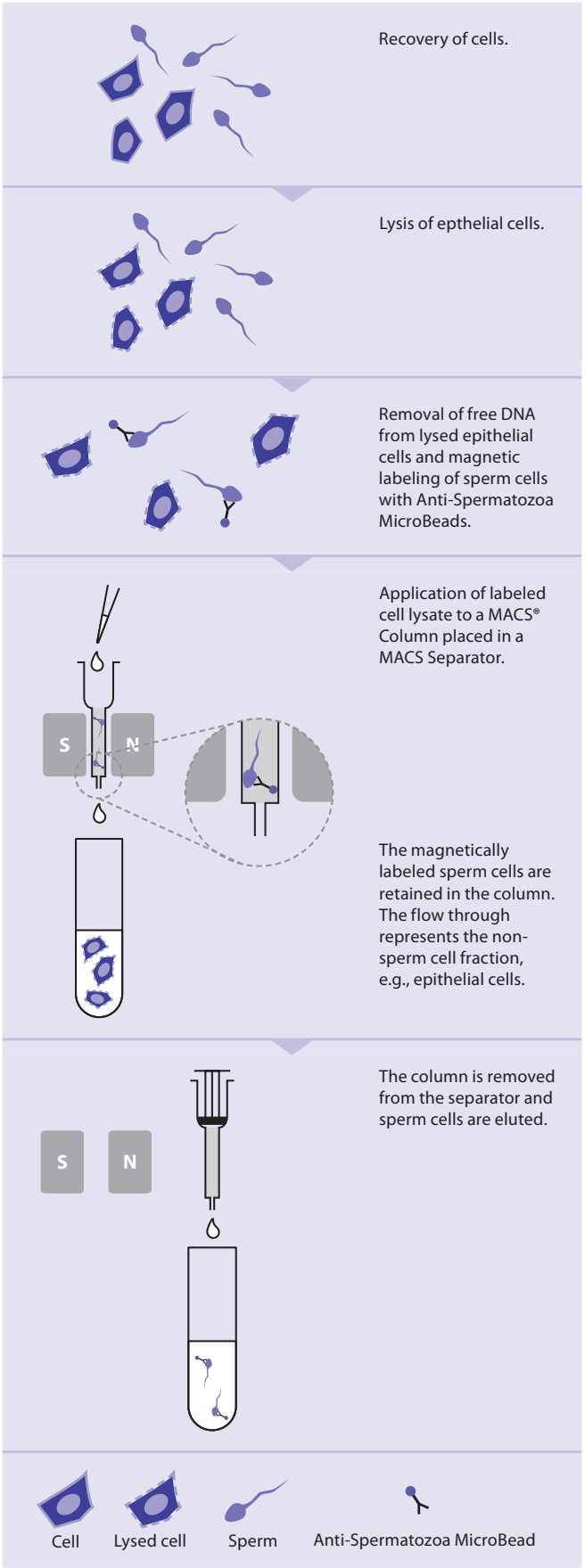
2. Protocol

2.1 Overview



2.2 Reagent preparation upon arrival for storage

1. Prepare Enzyme A by reconstitution of the lyophilized powder in the vial with 1 mL of Buffer A (supplied with the kit). Do not vortex. Prepare aliquots of appropriate volume to avoid repeated freeze-thaw-cycles, e.g. 40 µL/aliquot. Store aliquots at –20 °C. This Solution is stable for 6 months.
2. Preparation of 1× Free-DNA Removal Buffer by diluting Free-DNA Removal Buffer (10×) with distilled water 1:10: Mix 36 mL distilled water with 4 mL of Free-DNA Removal Buffer (10×) and store at 4 °C. Buffer can be stored for 6 months.

2.3 Before starting

▲ Cool down centrifuge to 4 °C.

▲ Adjust Thermomixer to 21-25 °C.

▲ Prepare solutions 1 and 2.

Preparation of Solution 1

Prepare directly before use: Add dissolved proteinase K to MACSprep™ Forensic Buffer to a final concentration of 100 µg/mL. Mix well and keep at 4 °C.

▲ **Note:** For each swab sample, 700 µL of Solution 1 is needed.

▲ **Note:** When using ready-to-use proteinase K, e.g. 20 mg/mL, add 5 µL of proteinase K to 955 µL MACSprep Forensic Buffer for 1mL of Solution 1.

Preparation of Solution 2

Prepare directly before use: Add 2 µL Enzyme A to 60 µL 1× Free-DNA Removal Buffer. Mix well and keep at 4 °C.

▲ **Note:** For each swab sample, 60 µL of Solution 2 is needed.

Solution	Component (final volume 1 mL)	Volume for one swab sample
Solution 1	5 µL Proteinase K (20 mg/mL) 955 µL MACSprep Forensic Buffer	700 µL
Solution 2	2 µL Enzyme A 60 µL 1× Free-DNA Removal Buffe	60 µL

2.4 Sample preparation

1. Transfer the head of a swab into a 1.5 mL tube (tube A).

   ▲ **Note:** For cotton swab sample: Remove cotton material from swab and cut into small pieces.
2. Add 600 µL Solution 1.
3. Vortex for 10 seconds.
4. Incubate for 30 minutes at 25 °C on a thermomixer with 800 rpm mixing.
5. Vortex for 10 seconds and spin down briefly to remove any drops from inside of the lid.
6. Place a spin basket into a new 1.5 mL tube (tube B).
7. Transfer the swab head and the solution from tube A to the spin basket in tube B.
8. Add 100 µL Solution 1 to the tube A to rinse the tube.
9. Transfer the rinsing onto the swab head within the spin basket in tube B.

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10. Centrifuge tube B at 13,000 rpm (16,000×g) for 5 minutes at 4 °C.
11. Discard the spin basket and swab head.
12. Collect supernatant to a new 1.5 mL tube without disturbing the pellet.

    ▲ **Note:** Keep the supernatant for further downstream applications. It contains non-spermatozoa material, e.g. epithelial cells.

    ▲ **Note:** The volume of supernatant after centrifugation might range from 500 µL to 650 µL depending on the type of swabs.
13. The pellet is needed to proceed with chapter 2.5.

2.5 Removal of free DNA and magnetic labeling

1. Resuspend the pellet with 60 µL Solution 2.
2. Add 40 µL of Anti-Spermatozoa MicroBeads.
3. Mix well and incubate for 15 minutes at 25 °C without agitation.
4. Directly proceed with chapter 2.6.

2.6 Magnetic separation

▲ Choose an MS Column and an appropriate MACS® Separator. For details refer to the table in section 1.4.

▲ Always wait until the column reservoir is empty before proceeding to the next step.

Magnetic separation with MS Columns

1. Place MS Column in the magnetic field of a suitable MACS Separator. For details refer to the MS Column data sheet.
2. Prepare column by rinsing with 500 µL of MACSprep™ Forensic Buffer.
3. Apply 100 µL cell suspension (from chapter 2.5) directly on a MS Column. Collect flow-through containing unlabeled cells.

   ▲ **Note:** Avoid spilling the cell suspension onto the column wall. Apply the cell suspension directly onto the column.
4. To recover residual material from the empty 1.5 mL tube (from chapter 2.5): Add 400 µL MACSprep Forensic Buffer to the tube and then apply it to the MS Column. Collect flow-through containing unlabeled cells and combine with the flow-through from step 3.
5. Wash column with the 3×500 µL of MACSprep Forensic Buffer. Collect unlabeled cells that pass through and combine with the flow-through from steps 3 and 4.

   ▲ **Note:** Perform washing steps by adding buffer aliquots as soon as the column reservoir is empty.
6. Remove column from the separator and place it on a new 1.5 mL tube.
7. Pipette 500 µL of MACSprep Forensic Buffer onto the column. Immediately flush out the magnetically labeled cells (spermatozoa) by firmly pushing the plunger into the column.
8. Centrifuge cell suspension at 13,000 rpm (16,000×g) for 5 minutes at 4 °C.
9. Aspirate supernatant carefully. The pellet is used for further downstream applications.

3. References

1. Yeung, C. H. *et al.* (1997) Human epididymal secreted protein CD52 on ejaculated spermatozoa: correlations with semen characteristics and the effect of its antibody. Mol. Hum. Reprod. 3(12): 1045–1051.

The use of the product in the method for separation of spermatozoa as described herein is covered by US patent no. 10060914B1.
The European patent application no. 3155428A1 is pending.

Refer to **www.miltenyibiotec.com** for all data sheets and protocols. Miltenyi Biotec provides technical support worldwide. Visit www.miltenyibiotec.com/local to find your nearest Miltenyi Biotec contact.

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