Miltenyi Biotec

# StraightFrom® Leukopak® CD14 MicroBead Kit
human

Order no. 130-117-020

## 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** | **4 mL StraightFrom® Leukopak® CD14 MicroBeads, human:**<br>MicroBeads conjugated to monoclonal anti-human CD14 antibodies (isotype: mouse IgG2a).<br>**1× 50 mL Whole Blood Column Elution Buffer**<br>**1× Multi-24 Column Block**<br>1× Multi-24 Column Block and 1× 24-well Deep Well Plate, sterile packed. |
| **Capacity** | For one ½ Leukopak®. |
| **Product format** | StraightFrom® Leukopak® CD14 MicroBeads are supplied in buffer containing stabilizer and 0.05% sodium azide.<br>Whole Blood Column Elution Buffer contains stabilizer and 0.09% sodium azide. |
| **Storage** | Store StraightFrom® Leukopak® CD14 MicroBeads and Whole Blood Column Elution Buffer protected from light at 2–8 °C. Do not freeze.<br>Store Multi-24 Column Block dry at 10–35 °C and protected from light.<br>The expiration date is indicated on the vial or box label. |

### 1.1 Principle of the MACS® Separation

First, the CD14$^+$ cells in a Leukopak® sample are magnetically labeled with StraightFrom® Leukopak® CD14 MicroBeads. Then, the cell suspension is loaded onto a Multi-24 Column Block, which is placed in the magnetic field of a MultiMACS™ Cell24 Separator Plus. The magnetically labeled CD14$^+$ cells are retained within the column. The unlabeled cells run through; this cell fraction is thus depleted of CD14$^+$ cells. After removing the column from the magnetic field, the magnetically retained CD14$^+$ cells can be eluted as the positively selected cell fraction.

### 1.2 Technical specifications of the Multi-24 Column Block

One Multi-24 Column Block is a unit of 24 columns, enabeling up to 24 separations in parallel.

- Column capacity: $1\times10^8$ magnetically labeled cells from up to $1\times10^9$ total cells per single column.
- Columns are “flow stop” and do not run dry.
- Void volume per single column: 250 µL. Reservoir volume: 5 mL.

  ▲ **Note:** If sample volume exceeds 5 mL per column, apply sample in aliquots.
- Multi-24 Column Blocks are for single use only.

### 1.3 Background information

During leukapheresis white blood cells are separated from whole blood and collected as highly concentrated leukocytes in Leukopaks®, which are ideal for the isolation of large numbers of various leukocyte subsets.
The StraightFrom® Leukopak® CD14 MicroBead Kit has been developed for the positive selection of CD14$^+$ cells directly from a Leukopak® by using the MultiMACS Cell24 Separator Plus. No sample preparation is required, including density gradient centrifugation or erythrocyte lysis. The CD14 antigen belongs to the LPS receptor complex. It is strongly expressed on most monocytes and macrophages and weakly on neutrophils.

### 1.4 Applications

- Isolation of CD14$^+$ cells from Leukopaks®. The purified CD14$^+$ cells are well suited for further flow cytometric, functional, or molecular analysis.



**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**

### 1.5 Reagent and instrument requirements

- Separation buffer: Prepare a solution containing phosphate-buffered saline (PBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA by diluting MACS® BSA Stock Solution (# 130-091-376) 1:20 with autoMACS® Rinsing Solution (# 130-091-222). Keep buffer cold (2–8 °C). Alternatively, use autoMACS Running Buffer (# 130-091-221). Degas buffer before use, as air bubbles could block the column.

  ▲ **Note:** BSA can be replaced by other proteins such as human serum albumin, human serum, or fetal bovine serum (FBS). Buffers or media containing $Ca^{2+}$ or $Mg^{2+}$ are not recommended for use.

- MultiMACS™ Cell24 Separator Plus (# 130-098-637)
- 24-well Deep Well Plates (# 130-110-500) or Single-well Deep Well Plates (# 130-114-966)
- (Optional) Fluorochrome-conjugated antibodies for flow cytometric analysis, e.g., CD15-PE-Vio® 770, CD14-APC, and CD45-VioBlue®. For more information about antibodies refer to www.miltenyibiotec.com/antibodies.
- (Optional) Propidium Iodide Solution (# 130-093-233) or 7-AAD Staining Solution (# 130-111-568) for flow cytometric exclusion of dead cells.

## 2. Protocol

▲ The StraightFrom® Leukopak® CD14 MicroBead Kit has been developed for positive selection of target cells from one ½ Leukopak® with up to 200 mL ($5\times10^9$–$1\times10^{10}$ cells). When working with higher volumes per ½ Leukopak®, please contact our Technical Support team.

### 2.1 Preparation of Leukopak®

1. Transfer up to 200 mL of a ½ Leukopak® into a collection tube. Centrifuge at 200×g for 10 minutes and discard supernatant. Fill up to 100 mL with separation buffer and resuspend.
2. Proceed to magnetic labeling (2.2).

### 2.2 Magnetic labeling

▲ Work fast, keep cells cold, and use pre-cooled solutions. This will prevent capping of antibodies on the cell surface and non-specific cell labeling.

▲ Volumes for magnetic labeling given below are for one tube containing 100 mL sample. When working with smaller volumes, scale down all reagents and total volumes accordingly (e.g. for ¼ Leukopak®, fill up to 50 mL and use 2 mL StraightFrom® Leukopak® CD14 MicroBeads and half of the Multi-24 Column Block).

▲ The recommended incubation temperature is 2–8 °C. Higher temperatures and/or longer incubation times may lead to non-specific cell labeling. Working on ice may require increased incubation times.

1. Add 4 mL StraightFrom® Leukopak® CD14 MicroBeads to the tube containing 100 mL sample.
2. Mix well by inverting the tube and incubate for 15 minutes in the refrigerator (2–8 °C).
3. Proceed directly to magnetic separation (2.3).

### 2.3 Magnetic separation

▲ For more detailed instructions on how to use the MultiMACS Cell24 Separator Plus, please refer to the user manual.

▲ The MultiMACS Cell24 Separator Plus, including the MACS Elution Station, has to be used with a Multi-24 Column Block and three Deep Well Plates for magnetic separation with StraightFrom® Leukopak® CD14 MicroBeads.

▲ **Note:** To reach maximum cell recovery, rinse the Deep Well Plate after removal of positive fraction and combine with the positive fraction. For the collection of the negative fraction and the wash fractions, use two Deep Well Plates.

▲ Buffer volumes per column are as follows:

Equilibration: 2 mL (separation buffer)
Wash: 3×1 mL (separation buffer)
Elution: 1 mL (Whole Blood Column Elution Buffer)

▲ Divide the sample equally between the 24 columns of the Multi-24 Column Block, e.g., when starting volume is 104 mL (100 mL Leukopak® and 4 mL StraightFrom® Leukopak® CD14 MicroBeads), add 4.4 mL onto each column.

▲ Select the program **POSSEL2** and follow the on-screen instructions of the MultiMACS Cell24 Separator Plus.

▲ After the separation, centrifuge positive fraction at 200×g for 10 minutes. Aspirate supernatant carefully. Resuspend cell pellet in a suitable amount of buffer or medium for subsequent analysis.



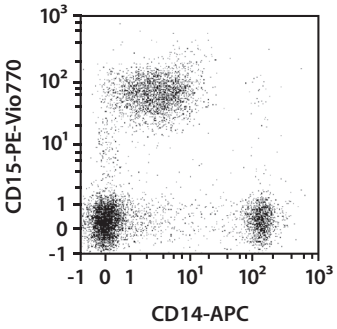


140-005-243.04

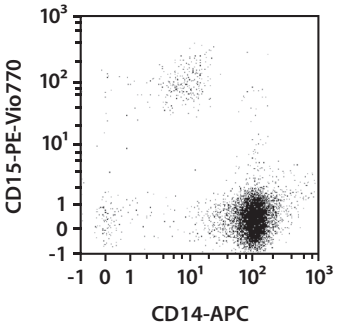
## 3. Example of a separation using the StraightFrom® Leukopak® CD14 MicroBead Kit

Separation of a Leukopak® sample using the StraightFrom® Leukopak® CD14 MicroBead Kit and the MultiMACS Cell24 Separator Plus with the Multi-24 Column Block. Cells were fluorescently stained with CD15-PE-Vio770, CD14-APC, and CD45-VioBlue and analyzed by flow cytometry using the MACSQuant® Analyzer. Cell debris and dead cells were excluded from the analysis based on scatter signals and propidium iodide fluorescence.

Before separation




CD14⁺ cells




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.

## 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

autoMACS, MACS, MACSQuant, the Miltenyi Biotec logo, MultiMACS, StraightFrom, Vio, and VioBlue are registered trademarks or trademarks of Miltenyi Biotec and/or its affiliates in various countries worldwide. All other trademarks mentioned in this publication are the property of their respective owners and are used for identification purposes only.

Leukopak is a registered trademark of StemExpress, LCC.