Miltenyi Biotec

MACSQuant® Tyto® Cartridges LC

8 Cartridges 130-133-260

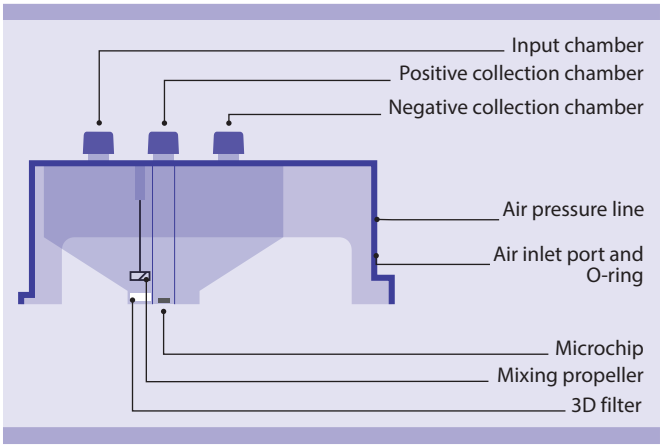
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1. Description

This product is for research use only.

Components	**8 MACSQuant Tyto Cartridges LC**, sterile and single packed, for sorting of larger cells (LC)
Storage	Store MACSQuant Tyto Cartridges LC dry and protected from light at room temperature (+10 to +25 °C). The expiration date is indicated on the box label. Do not use after this date.



Regulatory notes

The product is compliant with European Directive 2011/65/EU on the restriction of the use of certain hazardous substances in electrical and electronic equipment (RoHS).
The product is in scope of European Directive 2012/19/EU on waste electrical and electronic equipment (WEEE).

Important note for WEEE: Any product sent back for recycling must be free from contamination. If the product is suspected to be contaminated, a complete decontamination of the product has to be performed before sending back for recycling. In case of biological contamination where the product is expected to be infective, the product is not to be recycled but treatment and disposal of the product as biohazardous waste must be conducted according local regulations.

1.1 Background information

The closed, single-use disposable MACSQuant Tyto Cartridges LC have been designed for sorting larger cells (LC) with 20–50 µm in diameter using a MACSQuant Tyto Instrument with MACSQuantify™ Tyto Software version 3.2 or higher.
Their proprietary design enables cells to be aseptically administered and removed.
Once a cartridge is loaded into the MACSQuant Tyto Instrument, cell samples are driven with low, positive pressure through a sorting microchip. There, cells are optically analyzed/interrogated by passing three laser spots and sorted by a mechanical sort valve. After sorting, target and non-target cells can be removed from the cartridge for further downstream applications.

1.2 Principle of microchip-based sorting

Labeled cells are loaded into the input chamber of the MACSQuant Tyto Cartridge LC. A low air pressure is applied by the MACSQuant Tyto Instrument on the cells in the input chamber to drive them via a microchannel into the sorting microchip. Within the microchip, cells are interrogated by three lasers. Based on their fluorescent and scatter light signatures, target cells are identified.
Once a target cell is identified, a magnetic field is applied to the microchip. This triggers the sorting valve to open and therefore redirect the target cell into the positive collection chamber. After a target cell has passed through, a single-crystal silicon spring returns the valve into its original position. The default destination of non-target cells is the negative collection chamber.

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1.3 Technical specifications

- For single use only.
- Sample volume
  - 100 µL – 10 mL
  - Maximum of 1×10^7 cells/mL
- Dimensions
  - Depth: 50 mm
  - Width: 31.5 mm
  - Length: 95 mm
- Chamber capacities
  - Input chamber: 10 mL
  - Negative collection chamber: 10 mL
  - Positive collection chamber: 2 mL

1.4 Reagent and instrument requirements

- MACSQuant Tyto Instrument with MACSQuantify Tyto Software version 3.2 or higher, e.g., MACSQuant Tyto Cell Sorter (# 130-103-931)
- MACSQuant Tyto Priming Fixture (# 130-119-832)
- MACSQuant Tyto Running Buffer 1 × 100 mL (# 130-107-206) or 6 × 100 mL (# 130-107-207)
- Pre-Separation Filters (70 µm) (# 130-095-823)
- 0.2 µm inline disk filter
- 10 mL syringe with Luer-Lock tip
- Capillary pipet tips, sterile

2. Use of the MACSQuant Tyto Cartridges LC

▲ Please refer to the MACSQuant Tyto Instrument documents provided with the instrument for detailed information on using the instrument.

▲ Do not touch the microchip.

▲ For sterile sorts, all open cartridge steps must be performed in a biosafety cabinet.

2.1 Priming

▲ The following protocol is for manual priming. The MACSQuantify Tyto Software 3.2 or higher also includes an automated priming feature (refer to the MACSQuantify Tyto Software user manual).

1. Orient the cartridge so the owl icon is in the upper right corner and the caps appear in a ∴ shape.
2. Place the cartridge into the MACSQuant Tyto Priming Fixture by sliding the feet of the cartridge into the corresponding slots of the base. Start with the two slots on the right and lower into the two slots on the left. Verify that the cartridge is leveled out.
3. Fill 3 mL of the MACSQuant Tyto Running Buffer into a 10 mL syringe with a male Luer lock connection fitting.
4. Attach an 0.2 µm inline disk filter to the syringe. Discard 2 mL of buffer to flush the filter.
5. Remove cap from the input chamber of the cartridge and screw the syringe to the input chamber. Store the cap upside down.
6. Fill MACSQuant Tyto Running Buffer into the input chamber until the buffer level reaches the mixing propeller (~500 µL).
7. Unscrew the filter with syringe from the input chamber. Discard the filter and remaining buffer from the syringe.
8. Pull the syringe plunger out to its stop and screw the syringe back to the input chamber.
9. Press down on the upper left corner of the cartridge to block the air inlet port with O-ring. At the same time, push the plunger to let the MACSQuant Tyto Running Buffer flow into the positive collection chamber. Keep the pressure until buffer is visible in the positive collection chamber.
10. Remove the cartridge from the MACSQuant Tyto Priming Fixture.
11. Unscrew the syringe from the cartridge and pull the plunger out to its stop. Screw the syringe to the input chamber again.
12. Close the air inlet port with O-ring with your finger. Push the plunger of the syringe completely to flow the MACSQuant Tyto Running Buffer to the negative collection chamber. Hold the plunger down until the buffer level is just above the 3D filter.
13. Unscrew the syringe from the cartridge and unhand the air inlet port with O-ring.
14. (Optional) Remove the buffer from the input chamber by using the syringe. To avoid air entering the 3D filter, leave a layer of buffer above the 3D filter. Reconnect the cap to the input chamber. Remove the buffer from the positive and negative collection chambers by using a capillary pipet tip.
15. Proceed with sample loading (2.2).

2.2 Sample loading

1. Remove the plunger from a 10 mL syringe with a male Luer lock connection fitting. Store plunger upside down.
2. Remove cap from the input chamber and attach syringe. Store the cap upside down.

   ▲ **Note:** It has to be ensured that the mixing propeller within the input chamber of the cartridge is located to the lower end of the shaft.
3. Place a 70 µm nylon mesh filter (Pre-Separation Filters (70 µm), # 130-095-823) on top of syringe. Moisten filter with MACSQuant Tyto Running Buffer before use.

   ▲ **Note:** Do not place the cartridge on the priming fixture for sample loading.
4. Apply the cell suspension onto the filter to remove cell clumps.
5. (Optional) To increase the cell recovery, rinse the filter with MACSQuant Tyto Running Buffer.
6. Discard the filter. Save an aliquot (e.g. 500 µL) as original fraction for cell analysis purposes.
7. Carefully place the plunger on top of syringe.
8. Load the sample into the input chamber by firmly applying the plunger at a rate of 0.5 mL/s.

   ▲ **Note:** Do not load the cartridge with higher rate as cells might be lost in the negative collection chamber.
9. Remove the syringe and reconnect the cap to the input chamber.

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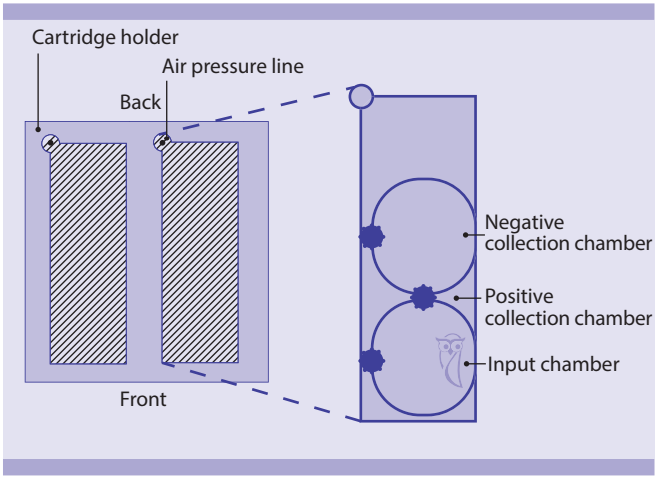
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10. Proceed with loading the MACSQuant Tyto Cartridge LC into the MACSQuant Tyto Instrument (2.3).

2.3 Loading the MACSQuant Tyto Cartridge LC into the MACSQuant Tyto Instrument

▲ For more information please refer to the MACSQuantify Tyto Software user manual.

1. Start the MACSQuant Tyto Instrument and MACSQuantify Tyto Software version 3.2 or higher and sign in.
2. Scan the 2D barcode on the MACSQuant Tyto Cartridge LC with the barcode scanner in the front of the instrument. The instrument door opens automatically upon scanning the cartridge.
3. Orient the cartridge so that the Tyto owl image is in the upright position. Place the cartridge in the right slot of the instrument stage. To insert the cartridge, first lower the front and then lower the back. Do not push the cartridge downward. Make sure that the O-ring is placed on the air pressure line of the instrument. The MACSQuant Tyto Cartridge LC is locked automatically. The door closes automatically shortly after inserting the cartridge. Instrument status indicator states blue.



4. For sort settings, please refer to the MACSQuantify Tyto Software user manual.
5. After the sort has been completed the MACSQuant Tyto Cartridge LC can be removed from the instrument and fractions can be removed (2.4).

2.4 Retrieval of cells from the MACSQuant Tyto Cartridges LC

For calculation of recovery and yield, the absolute cell number of each fraction (original, positive, and negative) has to be determined. Assess the cell concentration either using a cell counter or a MACSQuant Flow Cytometer.
Always start with the positive collection chamber.

2.4.1 Positive collection chamber

The target cells are contained in the positive collection chamber.

1. (Optional) When volume is <100 µL pipet MACSQuant Tyto Running Buffer into the positive collection chamber by using a capillary pipet tip.
2. Resuspend the sedimented cells by pipetting up and down.
3. Retrieve the full volume of cell suspension by using the pipet.
4. (Optional) For optimal retrieval wash the chamber with appropriate volume of MACSQuant Tyto Running Buffer.

2.4.2 Negative collection chamber

The non-target cells are contained in the negative collection chamber.

1. Invert or vortex the MACSQuant Tyto Cartridge LC to resuspend the sedimented cells.
2. Retrieve the needed volume of non-target cells from the negative collection chamber by using a capillary pipet tip. Alternatively, the complete cell sample can be retrieved using a syringe. Therefore, screw a syringe to the negative collection chamber and invert the MACSQuant Tyto Cartridge LC. Pull the syringe plunger out to its stop to retrieve the non-target cells.

3. Glossary of symbols

UKCA mark

CE mark (RoHS)

Waste electrical and electronic equipment

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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