METACELL

CIRCULATING RARE CELLS SEPARATION KIT

Product Data Sheet

Cat. No: EMC001

- Manufacturer: MetaCell, s.r.o., Czech Republic
- Use only the current version of Product Data Sheet enclosed with the kit!

1. PRINCIPLE OF THE METHOD

The MetaCell® kit is intended for a simple separation of circulating rare cells from peripheral blood (or other body fluids). MetaCell® technology uses a size-based separation principle. The following cell types may be enriched: circulating tumor cells (CTCs), disseminated tumor cells (DTCs), circulating tumor microemboli (CTM) and circulating endometrial cells (CECs).
As a result of the gentle flow of blood during the separation process, the enriched CTCs/ DTCs/ CTM/ CECs are in a very good viable condition. It is therefore possible to prepare a short-term (up to 5-7 days) *in vitro* culture of the enriched cells on a standard separation polycarbonate membrane.
The separation procedures are designed to enhance prognosis. A patient's response to treatment can be assessed by confirming the presence of circulating rare cells.

Note: Before use, all components of the MetaCell separation kit should be incubated under the UV – light for a minimum of 15 minutes to prevent external contamination. Special caution is required if long-term *in vitro* culture is planned and a prolonged time of incubation under UV is recommended.

2. STORAGE AND TRANSPORTATION CONDITIONS

Kit components may be transported and stored at temperatures ranging from 5 °C to 25 °C.

3. FEATURES

- Fast enrichment of intact CTCs/ DTCs/ CECs/ CTM (2-3 min) from peripheral blood (EDTA) and other bodily fluids (e.g. ascites, pleural effusion…)
- No problems related to coagulation during blood flow
- Isolation of both epithelial and mesenchymal tumor cells without the use of specific antibodies
- High detection capability – 1 tumor cell in 10 ml of blood
- Separation process does not require the use of any lysis reagents and the simple and gentle filtration does not affect the character of the cell
- Preservation of the morphological attributes of isolated cells
- Isolated cells can be counted immediately after separation process

- Enriched cells can be cultured *in vitro* for subsequent use/analysis/characterization
- No need for additional equipment for cell separation

4. PRECAUTIONS

- For professional use only
- MetaCell should be used under sterile conditions in a UV cabinet
- The product is not labelled as STERILE
- This product is intended for *in vitro* use only
- This product is not intended for self-testing
- This product is single-use only and not intended for repeated use
- If there is any damage to external packaging, check the integrity of kit components. If any components are broken or damaged, do not use the kit and contact your distributor

5. TECHNICAL HINTS

- Special training is not necessary, but is available if required
- Avoid any contamination between samples and reagents. Disposable PCR grade pipette tips with a filter should be used to pipette each sample and reagent
- Dispose of consumable materials and unused contents in accordance with applicable national regulatory requirements
- Special equipment is not required
- The device installation, maintenance and calibration are not required

6. KIT COMPOSITION

MetaCell® separation kit consists of a filling funnel, a plastic ring holding a separation membrane and a blood reservoir containing blood absorbents.

MetaCell® separation tube consists of filling funnel (1), which is accompanied by a plastic ring (2) holding a separation membrane (3), the tube and ring complex are fixed in a flown blood reservoir (4).

7. MATERIAL REQUIRED BUT NOT SUPLLIED

- EDTA pre-treated blood-withdrawal tube (e.g. Vacuette, Sarstedt, BD)
- RPMI 1640 medium (Note: for subsequent *in vitro* cultures RPMI must be supplemented with FBS and antibiotics)
- Microscopic slides and/ or 6-well plates
- NucBlue® Live ReadyProbes® Reagent and Celltracker™ Green CMFDA (Thermo Fisher Scientific, USA PCR tubes)
- TrypLE Select Enzyme (1X) (ThermoFisher)
- Fluorescence microscope
- Precision pipettes to deliver 0.5-1000 µl with disposable pipette tips

8. BLOOD COLLECTION AND HANDLING

The kit separates the circulating tumor cells from peripheral blood (PB). Collect approximately 8 ml of fresh venous blood into an EDTA pre-treated tube. Gently rotate the tube end-over-end five times immediately. The samples should be processed at room temperature within 24 hours from being taken from the patient.

9. CIRCULATING RARE CELLS SEPARATION FROM THE CLINICAL SAMPLE

Note: Before use, all components of the MetaCell separation kit should be incubated under the UV – light for a minimum of 15 minutes to prevent external contamination. Special caution is required if long-term *in vitro* culture is planned and a prolonged time of incubation under UV is recommended.

TRANSFERRING AND FILTRATION OF PERIPHERAL BLOOD (PB)

9.1. 8 ml of PB from patients is transferred into the filtration tube in the standard protocol.

Note:

- *The transfer of blood in several successive steps is preferable in order to prevent the blood clotting on the filter membrane. The PB flow through the filter is supported by capillary action.*

9.2. To start the filtration process, slightly push the plastic column to touch the absorbent layer, the blood will slowly flow through the membrane.

9.3. Ensure the full volume of blood has been filtered. The separation membrane will remain without blood after successful filtration and if there are no blood clots present on the membrane proceed to MEMBRANE WASHING.

Note:

- *In the case of blood clotting on the membrane during filtration process, apply the TrypLE solution on the filter to solubilize the clots (TrypLE Select Enzyme (1X), ThemoFisher; Blood : TrypLE = 1:1; max. volume of TrypLE 5 ml).*

MEMBRANE WASHING

The membrane is washed with RPMI after the blood filtration.

9.4. Wash the membrane by adding RPMI in a volume equal to 50% of the volume of the filtered blood. Allow the RPMI to flow through the membrane and repeat the washing twice (2X).

9.5. You may increase washing with RPMI up to 3X in case of redundant erythrocytes on the filter (red color).

MEMBRANE TRANSFER

9.6. Remove the tube out of the blue holder.

Note:

- *The blue reservoir containing the filtered blood may be discarded.*

9.7. Turn the tube slightly to loosen the plastic ring with the separation membrane.

9.8. For *in vitro* cultivation of the enriched cells: Place the plastic ring with the membrane into a 6- well plate.

9.9. Add growth medium (RPMI medium with FBS and antibiotics is recommended) to the well in the following manner to keep the cells on the separation membrane viable.

a. Add 1 ml of a cell culture medium to the bottom of the well.
b. Add 1 ml of a cell culture medium to the membrane space over the plastic ring (overlay the membrane).
c. Add 1 ml of a cell culture medium to the bottom of the well again.
d. Add 1 ml of a cell culture medium into the membrane space in the plastic (overlay the membrane).

10. IN VITRO CULTURE OBSERVATION

Two cell populations can be usually detected under the inverted light microscope after several days culture (3-5 days). *[See chapter 11 – Figures (A) and (B) referring May-Grünwald staining after the in vitro culture process.]*

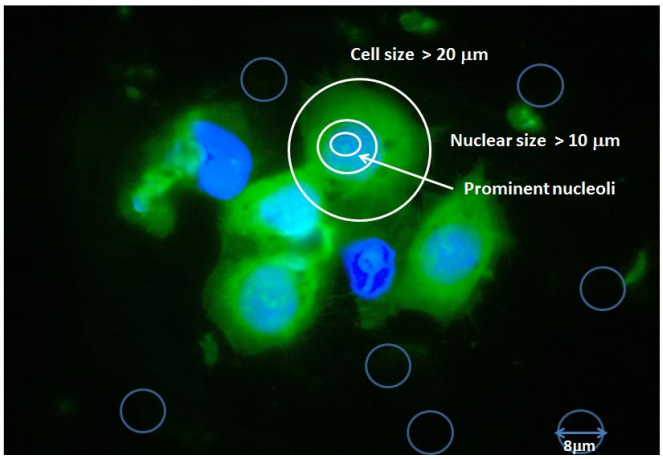
- the cells growing on the separation membrane (A).
- the cells which passed through the porous membrane and can be located growing on the bottom of the well (B).

» The membrane with captured cells may be stained by vital fluorescence stains after the short-term *in vitro* culture (3- 5 days), for example

- Cytoplasm stain (CellTracker™)
- Nuclear stain (NucBlue™) - staining procedure according manufacturer protocols (Thermo Fisher)

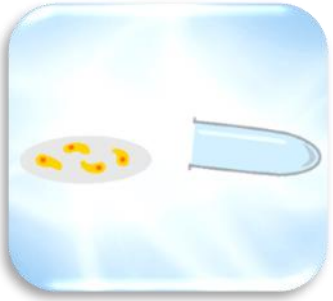
10.1. Wash the membrane in PBS before putting on a glass slide.

10.2. After short staining period (15 min) the separation membrane can be taken out of the plastic ring and put on a glass slide, cells may be observed under fluorescent microscope.



The cells trapped on the membrane stained fluorescently.

10.3. The cells can be cultivated further by placing the membrane back into the well with culture medium.
Note that working under strictly sterile conditions during preceding steps is crucial.
Alternatively, the membrane can be placed directly into an Eppendorf tube (1.5 ml) with RLT- BUFFER for subsequent RNA/ DNA analysis and stored at -20°C until use.

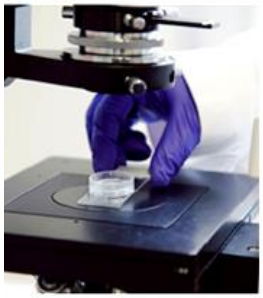
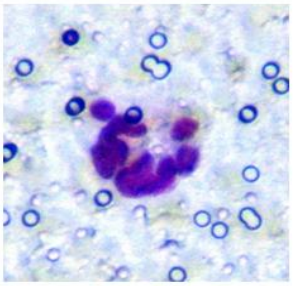


Note:

- *You may observe the cells on the membrane immediately after the separation process if needed.*

11. FIXATION, STAINING

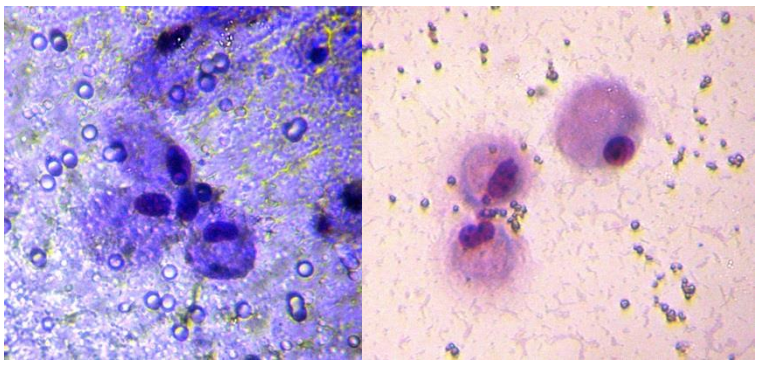
» If *in vitro* cultivation of the separated cells is not required, the membrane, kept in the plastic ring, may be transferred directly after the blood filtration to a standard glass microscope slide and stained.

The cells trapped on the membrane stained directly after the filtration process.

» Membrane with captured cells may be fixed and stained by May-Grünwald stain or any standard immunohistochemistry staining after the *in vitro* culture process.

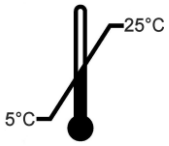
- the cells growing on the separation membrane (A).
- the cells which passed through the porous membrane and can be located growing in the bottom of the well (B).



(A) The cells trapped on the membrane.
(B) The cells on the bottom of the culture plate - MGG stained after 10 days of in vitro culture.

» For more information on this product see our webpages: www.metacell.cz

12. EXPLANATION OF SYMBOLS

REF	Catalogue number
Cont.	Content
LOT	Lot number
	See instructions for use
	Expiry date
	Storage conditions
	Identification of packaging materials

Distributed by

BioVendor GesmbH
Gaudenzdorfer Gürtel 43-45
1120 Vienna, Austria
Phone: +43 1 890 9025
Fax: +43 1 890 9025-15
E-mail: infoAustria@biovendor.com

BioVendor GmbH
Otto-Hahn-Straße 16
34123 Kassel, Germany
Phone: +49 6221 4339 100
Fax: +49 6221 4339 111
E-mail: infoEU@biovendor.com

www.biovendor.com

Oxford Biosystems Ltd
115J Olympic Avenue
Milton Park, Oxfordshire
OX14 4SA, United Kingdom
Phone: 01235 431390
E-mail: sales@oxfordbiosystems.com

www.oxfordbiosystems.com

Manufactured by

MetaCell

MetaCell s.r.o.
Erbenova 783/29
703 00 Ostrava, Czech Republic
Email: info@metacell.cz
www.metacell.cz