Monocyte Attachment Medium

PromoCell®

Instruction manual

Product	Size	Catalog number
Monocyte Attachment Medium	250 ml	C-28051

Recommended for

- Human mononuclear cells (freshly isolated)
- Human monocytes (freshly isolated)

Product description

Our Monocyte Attachment Medium allows for the efficient adherence selection of monocytes from freshly isolated human mononuclear cells while maintaining optimal cell health. Thus, the time-consuming and costly immunomagnetic purification of monocytes is dispensable prior to experiments requiring adherent monocytes as a starting material. The Monocyte Attachment Medium formulation is defined, animal-component-free, and protein-free. The medium does not need further supplementation.

Supplementation details

The Monocyte Attachment Medium contains all the growth factors and supplements necessary for providing optimal cell health and efficient adherence selection of monocytes from freshly isolated mononuclear cells. The Monocyte Attachment Medium does not contain antibiotics or antimycotics and is formulated for use in an incubator with an atmosphere of 5% CO_2.

Storage and stability

Store the medium at 2–8°C in the dark immediately after arrival. Do not freeze the medium. If stored properly, the product is stable until the expiry date stated on the label. After opening, the shelf life of the medium is 6 weeks at 2–8°C. For use, pre-warm only an aliquot of the medium and keep the remaining medium refrigerated at 2–8°C.

Quality control

All lots of PromoCell Monocyte Attachment Medium are subjected to comprehensive quality control tests using primary human mononuclear cells from peripheral blood. Each lot of Monocyte Attachment Medium is checked for the efficient adherence selection of highly viable monocytes. Approved in-house lots of media are used as a reference. In addition, all lots of media have been tested for the absence of microbial contaminants (fungi and bacteria) and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

Intended use

The products are for *in vitro* use only and not for diagnostic or therapeutic procedures. For safety precautions please see appropriate MSDS.

Instructions for the use of Monocyte Attachment Medium

The medium is ready-to-use and does not need further supplementation.

1

Isolate mononuclear cells

Isolate fresh mononuclear cells from blood samples using the Lymphocyte Separation Medium 1077 (C-44010) according to the protocol given in the instruction manual.

Note: Do not use buffy coats older than 20 hours, since this will significantly impair the experimental outcome. Buffy coats less than 8 hours old are optimal.

2

Analyze mononuclear cells

Count and analyze the isolated mononuclear cells for monocyte and granulocyte content (e.g., using the FSC/SSC plot of a flow cytometer). The granulocyte content should be less than 10% of the total mononuclear cells. Subsequently resuspend the cells at 100 million mononuclear cells per ml in Monocyte Attachment Medium.

Note: The adherence selection may be performed without prior analysis of the mononuclear cells (see step 3). However this might result in lower yield and purity due to suboptimal initial plating density and/or increased numbers of unwanted contaminating cells.

3

Allow the monocytes to attach

Plate freshly isolated mononuclear cells in an appropriate amount of pre-warmed Monocyte Attachment Medium, e.g., 15 ml medium per T-75 flask. Use a seeding density of 1 million/cm^2 for mononuclear cells with a monocyte content of ≥25% and 1.5 million/cm^2 for a monocyte content of <25%. Incubate for 1–1.5 hours at 5% CO_2 and 37°C in the incubator without any further manipulation.

Note: Use a plating density of 1.5 million mononuclear cells per cm^2 when mononuclear cells analysis in step 2 is skipped.

4

Wash the adherent cell fraction

Loosen non-adherent cells by vigorously swirling the tissue culture vessel and aspirate them. Thoroughly wash the adherent cells, i.e., monocytes, three times with pre-warmed Monocyte Attachment Medium by swirling the vessel and aspirating the supernatant.

Optional: Non-adherent cell fraction may be kept to isolate further blood cell types.

5

Use the adherent monocytes

Immediately after washing, add your medium of choice to the adherent monocytes. Use them for your experiments or continue the culture.

If you require special media modifications, we offer a custom media service starting at 10 bottles per order.
Contact us at info@promocell.com to find out more.

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01/2024