


Human Melanocyte Basal Medium (Calcium Separate)

Catalog Number M-254CF-500

Pub. No. MAN0001594 Rev. 3.0

 **WARNING!** Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Product description

Human Melanocyte Basal Medium, Calcium Separate (formerly Medium 254CF) is a sterile, liquid tissue culture medium intended for use as one component in a complete culture environment for the growth of normal human epidermal melanocytes. Human Melanocyte Basal Medium (Calcium Separate) is a modification of Medium 254, prepared without calcium chloride (CaCl₂). Human Melanocyte Basal Medium (Calcium Separate) is a basal medium containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, and inorganic salts. This medium does not contain antibiotics, antimycotics, hormones, growth factors, or proteins. This medium is HEPES and bicarbonate buffered and is designed for use in an incubator with an atmosphere of 5% CO₂/95% air.

To support plating and long-term proliferation of normal human melanocytes, Human Melanocyte Basal Medium (Calcium Separate) must be supplemented with calcium plus either Human Melanocyte Growth Supplement (HMGS) (Cat. No. [S0025](#)) or PMA-Free Human Melanocyte Growth Supplement (HMGS-2) (Cat. No. [S0165](#)). Each of these supplements contains all of the growth factors, hormones, and tissue extracts necessary for growth of melanocytes in Human Melanocyte Basal Medium (Calcium Separate). Sterile stock solution of calcium chloride (1000X; 0.2 M; 0.5 ml) is provided with each bottle of Human Melanocyte Basal Medium.

Contents and storage

Contents	Amount	Storage ^[1]
Human Melanocyte Basal Medium, Calcium Separate	500 mL	4°C; Protect from light; Do not freeze ^[2,3]
Calcium Chloride	0.5 mL ^[4]	Room temperature (18°C to 20°C)

^[1] When stored as indicated, all products are stable until the expiration date.

^[2] If the medium is warmed prior to use, do not exceed 37°C.

^[3] Once Medium has been supplemented with HMGS or HMGS-2, the supplemented medium should be stored in the dark at 4°C and is stable for 1 month.

^[4] Sterile stock solution of calcium chloride is 1000X and 0.2 M

Intended use

Human Melanocyte Basal Medium is intended for use in the routine culture of normal human epidermal melanocytes. When supplemented with HMGS or HMGS-2, Human Melanocyte Basal Medium will support the plating and proliferation of melanocytes at varying culture densities from 5 x 10³ cells/cm² to 1 x 10⁵ cells/cm². Additional applications for use may include primary isolation of melanocytes from skin.

- Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
- To add the calcium stock solution, determine the amount of calcium stock to be added. See equation and Table 1.

$$\frac{\text{Final mM concentration of CaCl}_2}{200 \text{ mM}} \times \text{mL of medium} = \text{mL of 0.2 M CaCl}_2$$

Prepare supplemented medium

CaCl₂ must be added to this medium prior to use. CaCl₂ from other sources is 0.5 μM in unsupplemented Human Melanocyte Basal Medium.

- Thaw one bottle of HMGS or one bottle of HMGS-2 according to the instructions provided with those products. Gently swirl the bottle of supplement.

Note: Make sure that the cap of the bottle is tight. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.

Table 1 Volume of 0.2 M CaCl₂ stock required

Final CaCl ₂ concentration	Volume of medium to be supplemented		
	100 mL	200 mL	500 mL
mM			
0.2	0.100	0.200	0.500
0.1	0.050	0.100	0.250
0.08	0.040	0.080	0.200
0.06	0.030	0.060	0.150
0.03	0.015	0.030	0.075

5. Add the stock solution to the medium dropwise, while slowly swirling the medium.

Note: Adding the calcium stock too fast may cause a precipitate.

6. (Optional) To add the HMGS or HMGS-2, transfer the entire contents of the bottle of supplement to the bottle of medium using sterile technique in a laminar flow culture hood.

7. Tightly cap the bottle of supplemented medium and swirl the contents to ensure a homogeneous solution.

Note: Avoid causing the medium to foam.

4. Using sterile technique in a laminar flow culture hood, draw up the stock solution in a 1 mL pipet.



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For descriptions of symbols on product labels or product documents, go to thermofisher.com/symbols-definition.

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Revision history: Pub. No. MAN0001594

Revision	Date	Description
3.0	14 April 2022	<ul style="list-style-type: none"> The product name and user manual title were updated. The user manual was updated to meet current style guidelines.

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