

Medium 254CF

(Calcium-Free*)

Cat. no. M-254CF-500

500 ml

Product Description

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. Medium 254CF is a modification of Medium 254, prepared without calcium chloride* for those investigators who desire to vary the concentration of calcium. Medium 254CF 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, Medium 254CF must be supplemented with calcium plus either Human Melanocyte Growth Supplement (HMGS, cat. # S-002-5) or PMA-Free Human Melanocyte Growth Supplement-2 (HMGS-2, cat. # S-016-5). Each of these supplements contains all of the growth factors, hormones, and tissue extracts necessary for growth of melanocytes in Medium 254CF. Sterile stock solution of calcium chloride (1000X; 0.2 M; 0.5 ml) is provided with each bottle of Medium 254CF.

Intended Use

Medium 254CF is intended for use in the routine culture of normal human epidermal melanocytes. When supplemented with HMGS or HMGS-2, Medium 254CF will support the plating and proliferation of melanocytes at varying culture densities from 5×10^3 cells/cm² to 1×10^5 cells/cm². Additional applications for use may include primary isolation of melanocytes from skin.

* **Calcium chloride must be added to this medium prior to use.** Calcium concentration from other sources is 0.5 µM in unsupplemented Medium 254CF.

This product is for research use only. Not for use in animals, humans, or diagnostic procedures.

Caution: If handled improperly, some components of this product may present a health hazard. Take appropriate precautions when handling this product, including the wearing of protective clothing and eyewear. Dispose of properly.

Storage and Stability

Medium 254CF is stored at 4°C in our facility and is shipped at ambient temperature. Upon receipt, the medium should be stored at 4°C and should not be frozen. **Protect from light.** Several components of this tissue culture medium are light-labile, and we recommend that the medium not be exposed to light for lengthy periods of time. If the medium is warmed prior to use, do not exceed 37° C. When stored in the dark at 4°C, the product is stable until the expiration date on the label.

Use the supplementation instructions (page 2) to prepare the medium for use.

For research use only.

Life Technologies Corporation • 5791 Van Allen Way • Carlsbad • CA 92008 • Tel: 800.955.6288 • www.invitrogen.com • E-mail: tech_support@invitrogen.com

Preparation of Supplemented Medium 254CF

Note: For information on HMGS or HMGS-2, refer to the product sheets that accompany those products.

1. Thaw one bottle of HMGS or one bottle of HMGS-2 according to the instructions provided with those products. Make sure that the cap of the bottle is tight. Gently swirl the bottle of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
2. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
3. To add the calcium stock solution, determine the amount of calcium stock to be added according to the table or formula below. Using sterile technique in a laminar flow culture hood, draw up the stock solution in a 1 ml pipet. Add the stock solution to the medium dropwise, while slowly swirling the medium. Adding the calcium stock too fast may cause a precipitate.

4. 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.

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

Storage and Stability of Supplemented Medium 254CF

Once Medium 254CF has been supplemented with HMGS or HMGS-2, the supplemented medium should be stored in the dark at 4°C and should not be frozen. When stored in the dark at 4°C, the supplemented medium is stable for 1 month.

Final [CaCl ₂]	Volume of medium to be supplemented			Volume (ml) of 0.2M CaCl ₂ stock required
mM	100ml	200ml	500ml	
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	

Formula:

Vol. 0.2M CaCl₂ (ml) to add = Desired final [CaCl₂] (mM) / 200mM x Vol. Medium to be supplemented (ml)

Limited Use Label License No. 5: Invitrogen Technology

The purchase of this product conveys to the buyer the non-transferable right to use the purchased amount of the product and components of the product in research conducted by the buyer (whether the buyer is an academic or for-profit entity). The buyer cannot sell or otherwise transfer (a) this product (b) its components or (c) materials made using this product or its components to a third party or otherwise use this product or its components or materials made using this product or its components for Commercial Purposes. The buyer may transfer information or materials made through the use of this product to a scientific collaborator, provided that such transfer is not for any Commercial Purpose, and that such collaborator agrees in writing (a) not to transfer such materials to any third party, and (b) to use such transferred materials and/or information solely for research and not for Commercial Purposes. Commercial Purposes means any activity by a party for consideration and may include, but is not limited to: (1) use of the product or its components in manufacturing; (2) use of the product or its components to provide a service, information, or data; (3) use of the product or its components for therapeutic, diagnostic or prophylactic purposes; or (4) resale of the product or its components, whether or not such product or its components are resold for use in research. For products that are subject to multiple limited use label licenses, the terms of the most restrictive limited use label license shall control. Life Technologies Corporation will not assert a claim against the buyer of infringement of patents owned or controlled by Life Technologies Corporation which cover this product based upon the manufacture, use or sale of a therapeutic, clinical diagnostic, vaccine or prophylactic product developed in research by the buyer in which this product or its components was employed, provided that neither this product nor any of its components was used in the manufacture of such product. If the purchaser is not willing to accept the limitations of this limited use statement, Life Technologies is willing to accept return of the product with a full refund. For information on purchasing a license to this product for purposes other than research, contact Licensing Department, Life Technologies Corporation, 5791 Van Allen Way, Carlsbad, California 92008. Phone (760) 603-7200. Fax (760) 602-6500. Email: outlicensing@invitrogen.com.

©2009 Life Technologies Corporation. All rights reserved.

For research use only. Not intended for any animal or human therapeutic or diagnostic use.

For research use only.

Life Technologies Corporation • 5791 Van Allen Way • Carlsbad • CA 92008 • Tel: 800.955.6288 • www.invitrogen.com • E-mail: tech_support@invitrogen.com