



Medium 254CF

(Calcium-Free*)

Catalog Number: M-254CF-500

Instructions for storage and use

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.

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.

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

*Calcium concentration from other sources is 0.5 μM in unsupplemented Medium 254CF.

Preparation of Supplemented Medium 254CF

Note: For information on HMGS or HMGS-2, please 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.
5. 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] mM	Volume of medium to be supplemented			Volume (ml) of 0.2M CaCl ₂ stock required
	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)

Terms and Conditions of Sale

Cascade Biologics, Inc. (hereinafter, CBI) warrants that its products will perform according to the information provided in various publications that it distributes, and as described herein for the intended shelf life of the product when stored under the conditions prescribed by CBI. If any product does not perform according to the published information provided by us, CBI will replace the product free of charge to the original customer. The remedy of product replacement shall be the customers' sole and exclusive remedy for defective product, unless CBI is unable to deliver replacement product, in which case CBI shall reimburse the customer for the purchase price of the defective product. Customer understands that the foregoing limited warranty is in lieu of all other warranties and CBI hereby disclaims all other warranties including, but not limited to, implied warranty of merchantability or fitness for adequacy for any particular purpose or use. CBI shall not be liable to customer or to any party claiming through customer for any incidental or consequential damages, including, but not limited to any lost profits, lost savings, or lost business, whether arising out of contract, tort, or otherwise. Customer acknowledges that products are intended for research use only and are not to be used in animals, humans, or diagnostic procedures.