



Medium 154XP

Catalog Number: M-154XP-500
500 ml

Instructions for storage and use

Product Description

Medium 154XP is a sterile, liquid tissue culture media intended for use as one component in a complete culture environment for the growth of normal human epidermal keratinocytes. Medium 154XP is a modification of Medium 154, prepared without calcium chloride*, thymidine, methionine, and cysteine for those investigators who desire to vary the concentrations of these components. Medium 154XP 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 keratinocytes, Medium 154XP must be supplemented with calcium, thymidine, methionine, and cysteine (provided with each bottle), plus either Human Keratinocyte Growth Supplement (HKGS, Cat. S-001-5) or Human Keratinocyte Growth Supplement Kit (HKGS Kit, Cat. S-001-K).

Sterile stock solutions of calcium chloride (1000x; 0.2 M; 0.5 ml), thymidine (1000x; 3 mM; 0.5 ml), methionine (1000x; 0.15 M, 0.5 ml), and cysteine (1000x; 0.24 M; 0.5 ml) are provided with each bottle of Medium 154XP.

*Calcium concentration from other sources is 0.5 μM in unsupplemented Medium 154XP.

Intended Use

Medium 154XP is intended for use by investigators who wish to vary the concentration of calcium, thymidine, methionine, or cysteine in cultures of normal human epidermal keratinocytes. When supplemented with calcium, thymidine, methionine, and cysteine, plus HKGS or HKGS Kit, Medium 154XP will support the plating and proliferation of keratinocytes at varying culture densities from clonal (25 cells/cm²) to high density (8 x 10⁴ cells/cm²). Additional applications for use may include primary isolation of keratinocytes from skin, and clonal growth assays. For optimal results when performing primary isolations, Medium 154XP should be used in conjunction with Coating Matrix Kit (Cat. R-011-K). ***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 154XP and the stock solutions are stored at -20° C in our facility and are shipped at ambient temperature. Upon receipt, the product may be partially thawed. Upon receipt, the medium and the stock solutions should be stored at -20° C in a freezer that is not self-defrosting. **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 -20° C, the product is stable until the expiration date on the label.

Please use the instructions on page 2 to prepare the medium for use.

Preparation of Medium 154XP

With each 500 ml bottle of Medium 154XP, we provide concentrated stock solutions of calcium (blue-capped vial), thymidine (yellow-capped vial), methionine (green-capped vial), and cysteine (red-capped vial). The stock solutions are sterile and ready for use. To supplement one bottle of Medium 154XP with the appropriate growth supplement (sold separately), and with calcium, thymidine, methionine, and cysteine at the concentrations found in Medium 154, we recommend the following protocol:

Note: For information on HKGS or HKGS Kit, please refer to the product sheets that accompany those products.

1. Thaw one bottle of Medium 154XP plus one tube each of calcium, thymidine, methionine, and cysteine stocks in a 37° water bath or overnight at 4° C. Remove each item from the water bath immediately upon thawing, and do not exceed 37° C. Check the Medium 154XP cap to make sure it is tight and swirl the bottle vigorously to ensure a homogenous solution.
2. Thaw the frozen components of the HKGS Kit, or one bottle of HKGS according to the instructions provided with those products. Make sure that the caps of all of the bottles are tight. Gently swirl the bottle(s) of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
3. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol.
4. Using sterile technique in a laminar flow culture hood, add the desired amount of the thymidine stock solution (yellow-capped vial), methionine stock solution (green-capped vial), and cysteine stock solution (red-capped vial) to the bottle of Medium 154XP. Cap the bottle of medium and swirl to ensure a homogeneous solution.
5. To add the calcium stock solution, determine the amount of calcium stock to be added. 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. *Note: addition of less than the entire amount of any stock solution may affect the performance of the supplemented medium*

- 6a. To add the HKGS, transfer the entire contents of the bottle of supplement to the bottle of medium using sterile technique in a laminar flow culture hood.
- 6b. To add the HKGS Kit, transfer the desired amount of each component of the HKGS Kit to the bottle of medium using sterile technique in a laminar flow culture hood. *Note: addition of less than the entire amount of any component may affect the performance of the supplemented medium. If antibiotics/antimycotics are desired, add the antibiotic/antimycotic solution included in HKGS Kit using the same technique as above.*
7. 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 154XP

Once Medium 154XP has been supplemented with HKGS or HKGS Kit, 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.

Selected References

The Medium 154XP formulation is based on medium MCDB 151, with trace elements as in medium MCDB 104, and the high amino acid modifications of Pittelkow.

Cook, Pittelkow, and Shipley; J. Cell. Physiol. 146:277-289, 1991
Peehl and Ham; In Vitro 16: 526-540, 1980
McKeehan et al.; In Vitro 13: 399-416, 1977
Pittelkow and Scott; Mayo Clin. Proc. 61: 771-777, 1986

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.