Cascade Biologics™
invitrogen cell culture

GIBCO®
invitrogen cell culture

Medium 154CF
(Calcium-Free*)

Cat. no. M-154CF-500
500 ml

Product Description

Medium 154CF and Medium 154CF/PRF are 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 154CF is a modification of Medium 154, prepared without calcium chloride* for those investigators who desire to vary the concentration of calcium. Medium 154CF/PRF is a phenol red-free version of Medium 154CF. Medium 154CF and Medium 154CF/PRF are basal media containing essential and non-essential amino acids, vitamins, other organic compounds, trace minerals, and inorganic salts. These media do not contain antibiotics, antimycotics, hormones, growth factors, or proteins. These media are HEPES and bicarbonate buffered and are designed for use in an incubator with an atmosphere of 5% CO_2/95% air. To support plating and long-term proliferation of normal human keratinocytes, Medium 154CF and Medium 154CF/PRF must be supplemented with calcium 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 solution of calcium chloride (1000x; 0.2 M; 0.5 ml) is provided with each bottle of Medium 154 CF and Medium 154CF/PRF.

*__Calcium chloride must be added to this medium prior to use.__ Calcium concentration from other sources is 0.5 µM in unsupplemented Medium 154CF and Medium 154CF/PRF

Medium 154CF/PRF
(Calcium- and Phenol Red-Free*)

Cat. no. M-154CF/PRF-500
500 ml

Intended Use

Medium 154CF is intended for use by investigators who wish to vary the concentration of calcium in cultures of normal human epidermal keratinocytes. Medium 154CF/PRF is intended for use by investigators who wish to culture normal human keratinocytes in the absence of phenol red. When supplemented with calcium plus HKGS or HKGS Kit, Medium 154CF and Medium 154CF/PRF will support the plating and proliferation of keratinocytes at varying culture densities from clonal (25 cells/cm^2) to high density (8 x 10^4 cells/cm^2). Additional applications for use may include primary isolation of keratinocytes from skin, and clonal growth assays. For optimal results when performing primary isolations, Medium 154CF and Medium 154CF/PRF 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 154CF, Medium 154CF/PRF and the calcium stock solution are stored at 4° C in our facility and are shipped at ambient temperature. Upon receipt, these media should be stored at 4° C and should not be frozen. **Protect from light.** Several components of these tissue culture media are light-labile, and we recommend that the media not be exposed to light for lengthy periods of time. If the media are 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.

Follow the instructions on page 2 to prepare the media for use.

Preparation of Supplemented Medium 154CF and Medium 154CF/PRF

With each 500 ml bottle of Medium 154CF and Medium 154CF/PRF, we provide a concentrated (0.2 M) stock solution of calcium chloride. The stock solution is sterile and ready for use. To supplement one bottle of either medium with calcium and the appropriate growth supplement (sold separately), we recommend the following protocol:

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

1. 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.
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 on the next page. 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 HKGS, transfer the entire contents of the bottle of supplement to the bottle of medium using sterile technique in a laminar flow culture hood. 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.
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 154CF and Medium 154CF/PRF

Once Medium 154CF or Medium 154CF/PRF 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 154CF and Medium 154CF/PRF formulations are 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

Final [$CaCl_2$]	Volume of medium to be supplemented			
mM	**100ml**	**200ml**	**500ml**	
0.2	0.100	0.200	0.500	**Volume (ml) of 0.2M $CaCl_2$ stock required**
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 $CaCl_2$ (ml) to add = Desired final [$CaCl_2$] (mM) / 200mM x Vol. Medium to be supplemented (ml)