Cascade Biologics™

invitrogen cell culture



EpiLife® Medium (Calcium-Free*)

Cat. no. M-EPICF-500 500 ml

Product Description

EpiLife® Medium Calcium-Free and EpiLife® Medium Calcium- and Phenol Red-Free 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 and normal human corneal epithelial cells. EpiLife® Medium Calcium-Free is a modification of EpiLife® Medium, prepared without calcium chloride* for those investigators who wish to vary the concentration of calcium. EpiLife® Medium Calcium- and Phenol Red-Free is a phenol red-free version of EpiLife® Medium Calcium-Free. These media 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% CO₂/95% air. To support plating and long term proliferation of human keratinocytes or human corneal epithelial cells, these media must be supplemented with calcium plus selected hormones and growth factors. We offer a choice of growth supplements for culturing human keratinocytes and human corneal epithelial cells. Please refer to the Table on page 2 to determine the best supplement choice for your purposes. The supplements contain all of the growth factors, hormones, and extracts necessary for growth of human keratinocytes or human corneal epithelial cells in EpiLife® Medium. Sterile stock solution of calcium chloride (1000x; 0.06 M; 0.5 ml) is provided with each bottle of EpiLife® Medium Calcium-Free and EpiLife® Medium Calcium- and Phenol Red-Free.

EpiLife® Medium (Calcium- and Phenol Red-Free*)

Cat. no. M-EPICF/PRF-500 500 ml

Intended Use

EpiLife® Media are intended for use in the routine culture of human epidermal keratinocytes or human corneal epithelial cells for research purposes. Additional applications for use may include primary isolation of keratinocytes or corneal epithelial cells. Refer to the table on page 2 for more information. *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

EpiLife[®] Media and the calcium stock solutions 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 <u>not</u> 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 supplementation instructions (pages 2-3) to prepare the medium for use

* Calcium chloride <u>must</u> be added to this medium prior to use. Calcium concentration from other sources is 0.65 µM in unsupplemented EpiLife[®].

EpiLife[®] is a Registered Trademark of Life Technologies.

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Preparation of Supplemented EpiLife® Medium

Depending on your application, obtain one of the growth supplement products shown in the table above (growth supplements sold separately). With each 500 ml bottle of EpiLife® Medium *Calcium-Free* and EpiLife® Medium *Calcium- and Phenol Red-Free*, we provide a concentrated (0.06 M) stock solution of calcium chloride. The stock solution is sterile and ready for use. To supplement one bottle of medium with calcium and the appropriate growth supplement, we recommend the following protocol:

- Thaw the chosen growth supplement according to the instructions provided with that product. Make sure that the caps of all of the bottles are tight.
- 2. Gently swirl the bottle(s) of supplement. Avoid splashing the supplement into the cap of the bottle or causing the supplement to foam.
- Obtain one bottle of medium with its attached tube of calcium chloride solution. Wipe the outside of the containers with a disinfecting solution such as 70% ethanol or isopropanol and move the containers to a laminar flow culture hood.
- 4. Using sterile technique, draw up the 0.5 ml of calcium 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: For optimal growth of keratinocytes and corneal epithelial cells in culture, we recommend that the entire 0.5 ml contents of the tube of 0.06 M calcium chloride be added to a 500 ml bottle of medium. Doing so will result in a calcium concentration in EpiLife® of 0.06 mM.

- 5. To add the EDGS, Supplement S7, HKGS or HCGS, use sterile technique in a laminar flow culture hood and transfer the entire contents of the bottle of supplement to the bottle of medium. To add the HKGS Kit, use sterile technique and transfer the desired amount of each component of the HKGS Kit to the bottle of medium. 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.
- 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 EpiLife®

Once EpiLife[®] medium has been supplemented with EDGS, Supplement S7, HKGS, HKGS Kit, or HCGS, the supplemented medium should be stored at 4° C and should not be frozen. When stored at 4° C, the supplemented medium is stable for 1 month.

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EpiLife [®] Extended-Lifespan Culture Systems				
	For keratinocytes:			For corneal epithelial cells:
	Defined, APF	Defined	Undefined	Undefined
Basal Medium	EpiLife [®] Medium (M-EPIcf-500, M-EPIcf/PRF-500, or M-EPI-500-CA)	EpiLife [®] Medium (M-EPIcf-500, M-EPIcf/PRF-500, or M-EPI-500-CA)	EpiLife [®] Medium (M-EPIcf-500, M-EPIcf/PRF-500, or M-EPI-500-CA)	EpiLife [®] Medium (M-EPIcf-500, M-EPIcf/PRF-500, or M-EPI-500-CA)
Growth Supplement	Supplement S7* (S-017-5)	EDGS** (S-012-5)	HKGS (S-001-5) or HKGS Kit (S-001-K)	HCGS (S-009-5)
Coating Matrix Kit	Coating Matrix Kit (R-011-K)	Coating Matrix Kit (R-011-K)	NA	N/A
Subculture Reagent	Recombinant Trypsin/EDTA (R-009-50)	Trypsin/EDTA (R-001-100) or Recombinant Trypsin/EDTA (R-009-50)	Trypsin/EDTA (R-001-100)	Trypsin/EDTA (R-001-100)
Subculture Reagent	Defined Trypsin Inhibitor (R-007-100)	Defined Trypsin Inhibitor (R-007-100)	Trypsin Neutralizer (R-002-100)	Trypsin Neutralizer (R-002-100)

^{*}For optimal cell growth, Supplement S7 must be used in conjunction with EpiLife® Medium and Coating Matrix Kit.

Limited Use Label License No. 5: Invitrogen Technology

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^{**}For optimal cell growth, EDGS should be used in conjunction with Coating Matrix Kit and Defined Trypsin Inhibitor.