

Lung Cancer Associated Fibroblast Media

HLCAF.E.Media-450

Media Usage Protocol:

Lung Cancer Associated Fibroblast Expansion Media is designed to be used with Human Lung Cancer Associated Fibroblasts, which are available separately. When used as directed, this media will support vigorous growth of these cells. The following is the recommended protocol for the usage of this media.

Note: Once complete media has been formulated, it should be stored at 4°C. Avoid extended exposure of the medium to room or higher temperatures. Medium should be equilibrated in a water bath set at 37°C before adding to any cell culture.

Additional Reagents Needed

1. Fetal Bovine Serum, High Grade or Characterized. Store in aliquots of 50 mL at -20°C.
2. Penicillin/Streptomycin/Amphotericin B solution, 100X or Penicillin/Streptomycin solution, 100X. These solutions should be portioned in 5 mL aliquots, stored at -20°C and never freeze/thawed. Although anti-mycotics are not absolutely necessary, CET highly recommends their usage for long term cell culture.

CET has no recommendation as far as a specific vendor for these products but urges investigators to use the highest grade of reagents available for best results.

Formulating Complete Lung Cancer Associated Fibroblast Expansion Media

1. Defrost 50 mL of fetal bovine serum and 5 mL of antibiotic/antimycotic solution in a 37°C water bath until ice in the tubes is no longer visible.
2. Immediately disinfect the tubes and the bottle containing the base media with 70% isopropanol.
3. Working in a laminar flow hood, remove 5 mL of the media from the bottle and discard. This and all other procedures must be done in a sterile manner.
4. Add 50 mL of the fetal bovine serum to the base media.
5. Add 5 mL of the antibiotic/antimycotic solution to the base media.
6. Cap the bottle containing the now complete media and gently swirl a few times. The complete media is now ready to use.
7. For any cell based applications, pre-warm the complete media to 37°C before use. Store complete media at 4°C when not in use.
8. As a general rule, cells should be fed with fresh, complete media every 72 hours and old media should be discarded before new complete media is added.

Table 1. Preparation of 500mL complete Lung Cancer Associated Fibroblast Expansion Media

Brand	Amount For 500mL	Product	Catalog #
CET	450mL	CET Lung Cancer Associated Fibroblast Expansion media	HLCAF.E.Media-450
Any	50mL	Fetal Bovine Serum	Refer to Manufacturer's Catalog Number

Store at 4°C.



Figure 1: Lung Cancer Associated Fibroblasts Media

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Note: Antibiotics/ antimycotics should not be used as an alternative to proper aseptic technique.



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Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell markers. All other cells are evaluated either by staining, method of isolation or traditional molecular biology techniques. Data is available upon request.

All growth and differentiation media are evaluated by conducting assays to make sure cells either grow or differentiate as indicated on the media label. Data is available upon request.

All cells are tested for HIV-1, HIV-2, Hepatitis B and Hepatitis C using sensitive PCR based assays. All cells test negative for these viruses. However, all human cells must be used in accordance with established laboratory safety procedures and only under the supervision of trained personnel.

All products are for research use only. Not for diagnostic or therapeutic use. CET's products are designed and tested to function with other CET products only. For example, all of our cells are optimized to grow and differentiate in CET media. Although investigators are welcome to formulate their own media, CET cannot and will not guarantee that cells will function as indicated in the product brochure. Moreover, such third party use will void CET's obligation to replace cells, should they not function as indicated.



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