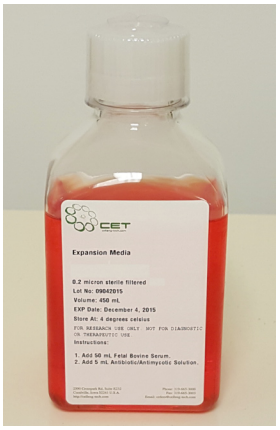
iPS Growth Media Kit

CET.IPS.GMK-500



Media Usage Protocol:
CET's iPS Growth Media is a universal, defined media for the expansion of human iPS cells. This media has been formulated and validated to be compatible with multiple substrates such as Vitronectin XF, Geltrex, Matrigel and Laminin. This provides more defined, cost effective and reproducible growth conditions. CET's iPS Growth Media has been uniquely formulated to restrict cell differentiation, promote robust growth and prevent cell death. 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. Shelf life of complete media is 14 days. Avoid extended exposure of the medium to room temperature or higher temperatures. Medium should be equilibrated at room temperature before addition to any cell culture.

Additional Reagents Needed:
1. 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 regarding a specific vendor for the above reagents, but urges investigators to use the highest grade of reagents available for best results.

Formulating Complete Induced Pluripotent Stem Cell Growth Media
1. Defrost iPS Growth Supplement at 4°C (the day before media is to be prepared) and 5 mL of antibiotic/antimycotic solution in a 37°C water bath until ice in the tubes is no longer visible. Never defrost IPS Growth Supplement using a 37°C water bath. It is normal for iPS Growth Supplement to appear hazy or have suspended solutes. Gently mix by inversion.
2. Immediately disinfect the tubes and the bottle containing the iPS Base Media with 70% isopropanol.
3. Working in a laminar flow hood, remove 12 mL of the iPS Base Media from the bottle and discard. This and all other procedures must be done in a sterile manner.
4. Add the complete contents of the iPS Growth Supplement to the iPS Base Media.
5. Add 5 mL of the antibiotic/antimycotic solution to the IPS Base Media.
6. Cap the bottle containing the now complete iPS Growth Media and gently swirl a few times. The complete media is now ready for use.
7. For any cell based applications, pre-warm the aliquot of complete media necessary to room temperature before use. Do not warm media in a water bath. Store the complete media at 4ºC when not in use. CET recommends using 2 mL of media per well of a 6-well dish and 10 mL of media per 100mm tissue culture dish.
8. As a general rule, cells should be fed with fresh complete media every 24 hours and old media should be discarded before new complete media is added.

Table 1. Preparation of 500 ml complete Induced Pluripotent Stem Cell Growth Media

Brand	Amount for 500 mL	Product	Catalog Number
CET	488 mL	CET Base Media	CET.IPS.GMK-500
CET	7 mL	CETGrowth Supplement	CET.IPS.GMK-500
Any	5 mL	Antibiotic/ Antimycotic solution	RefertoManufacturer's Catalog Number

Certificate of Analysis

All hematopoietic, mesenchymal and multipotent stem cells are evaluated by flow cytometry for specific stem cell mark-ers. 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 nega-tive 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.