PRIME-XV® AFSC Expansion Medium

Catalog # 91133

INTENDED USE

PRIME-XV® AFSC Expansion Medium is intended for use in the maintenance and expansion of human amniotic fluid stem cells (AFSCs) under ex vivo culture conditions while maintaining their ability to differentiate into different cell lineages. This medium is ready to use and can be supplemented with additional cytokine/ growth factors when desired for specific applications.

PRODUCT DESCRIPTION

PRIME-XV AFSC Expansion Medium is a serum containing medium for maintenance and expansion of human amniotic fluid stem cells. This product does not contain antibiotics.

SHIPPING

This product is shipped with dry ice. Upon receipt, store it immediately at the temperature recommended below.

STORAGE INSTRUCTIONS AND STABILITY

Upon receipt, store the medium at or below -10°C in a manual defrost freezer. Unopened medium is stable for 24 months from date of manufacture, as indicated on label, when stored at or below -10°C in a manual defrost freezer. PRIME-XV AFSC Expansion Medium can be aliquoted and stored at or below -10°C in a manual defrost freezer for up to 3 months. When ready to use, thaw this medium overnight at 2-8°C in the dark. PRIME-XV AFSC Expansion Medium should be used within one week when stored at 2-8°C and protected from light. Not validated for use beyond the unopened expiry shelf life. Repeated freeze-thaw cycles should be avoided.

PRECAUTIONS AND WARNINGS

For research use or further manufacturing use only. Not for injection or diagnostic procedures. The safety and efficacy of this product in diagnostic or other clinical uses has not been established. This reagent should not be used beyond the expiration date. Results may vary due to variations among human AFSCs derived from different donors.

Serum used in the production of PRIME-XV AFSC Expansion Medium has been tested for viral contamination per CFR Title 9 Part 113.53. It has also been screened for mycoplasma contamination. This product also contains transferrin derived from human plasma, which has been tested and found negative for antibodies to HIV-1/2, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV). However, the medium should be handled as if potentially infectious. Safe laboratory procedures should be followed and protective clothing should be worn when handing this medium. The acute and chronic effects of over-exposure to this medium are unknown.

RELATED PRODUCTS

Description	Catalog #	Size
PRIME-XV Osteogenic Differentiation SFM	91132	100 mL
PRIME-XV FreezIS	91139	10 mL, 100 mL

DIRECTIONS FOR USE

These procedures are general guidelines for culturing human AFSCs isolated from human amniocentesis. Procedures for optimal growth conditions should be determined for each application as appropriate.

- **Recovery of Cryopreserved Human AFSCs**

1. Thaw PRIME-XV AFSC Expansion Medium (Irvine Scientific, Catalog #91133) at 2-8°C for overnight or at room temperature. Pre-warm at 37°C the amount of medium needed for one procedure (repeated warming of medium may reduce product performance). Store the remaining medium at 2-8°C. Thawed medium can be kept in the refrigerator for up to one week.
2. Rapidly thaw a frozen vial of AFSCs in a 37°C water bath while swirling the vial until all its content is liquid. The vial should still feel cold to touch. The process typically takes less than 2 minutes.
3. Pipette the content of the entire vial into a 15 ml conical tube containing 5 to 10 ml pre-warmed PRIME-XV AFSC Expansion Medium.
4. Centrifuge the cells for 5 minutes at 300 xg at room temperature.
5. Re-suspend the pellet in pre-warmed PRIME-XV AFSC Expansion Medium (follow the instruction of subculture to determine the required volume of culture medium), count the cells and determine total viable cell number. Transfer the cell suspension to a appropriate culture flask for continuous culture.

- **Subculture of Human AFSCs in PRIME-XV AFSC Expansion Medium (Irvine Scientific Catalog # 91133)**

1. Aliquot the required quantity of PRIME-XV AFSC Expansion Medium and pre-warm the medium to 37°C.
2. Remove entire spent medium from culture flask and gently rinse the flask with equal volume of PBS without calcium and magnesium (Irvine Scientific, Catalog #9240).
3. Add pre-warm 1x Trypsin EDTA (Irvine Scientific, Catalog #9341) to the culture flask, 1mL for T-25 flask and 3mL for T-75 flask, and tilt the flask in all directions to disperse the Trypsin EDTA evenly over the cells.
4. Incubate the flask at 37°C, 5% CO_2 incubator for 5-10 minutes to detach the cells from culture surface. Tap the side of the flask to aid the detachment of the cells.
5. Add pre-warmed PRIME-XV AFSC Expansion Medium to the flask, 2mL for T-25 flask and 6mL for T-75 flask to stop trypsinizing. Disperse the cells by gently pipetting the media over the entire culture surface of the flask, and transfer the entire contents to a 15mL conical tube.
6. Centrifuge cells down at 300 xg for 5 minutes. Aspirate off supernantant.
7. Resuspend the cell pellet in a small amount of pre-warmed PRIME-XV AFSC Expansion Medium and count the cells with a cell counter.
8. Inoculate $1.25x10^5$ cells into T-25 flask containing 5mL pre-warmed PRIME-XV AFSC Expansion Medium, or $3.75x10^5$ cells into T-75 flask containing 15mL medium. Note: It is recommended to seed cells at approximately 5,000 cells/cm^2 of culture vessel.
9. Feed the cells by replacing the spent medium with equal amount of fresh pre-warm PRIME-XV AFSC Expansion Medium every 2 days.
10. Subculture the cells when the cell confluence reaches 70%. It usually takes 4 days if the seeding density at Step 8 is used.

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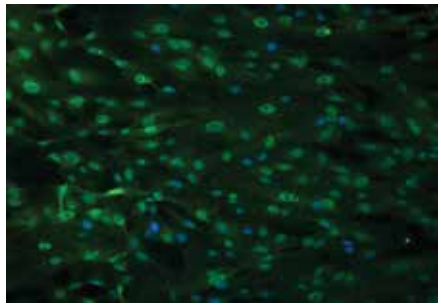
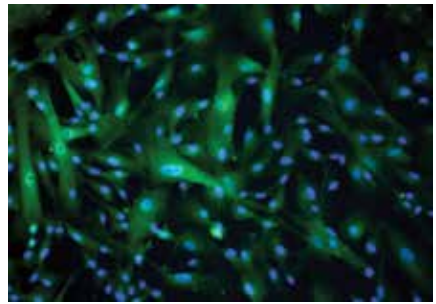
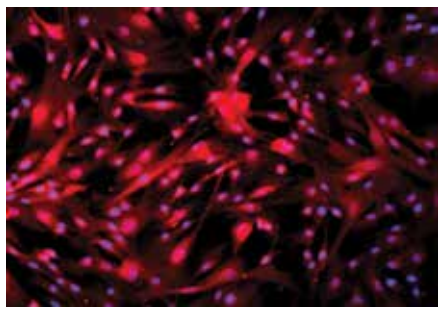
DATA



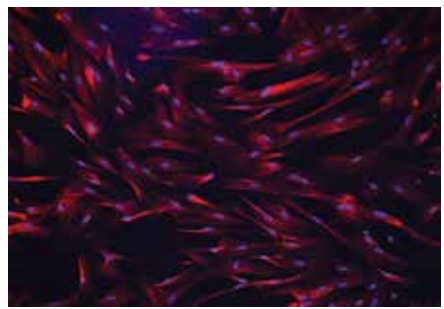
Immunofluorescence analysis of human AFSCs cultured in PRIME-XV AFSC Expansion Medium after six passages showed positive OCT4-A staining. Nuclei were counterstained with DAPI.



Immunofluorescence analysis of human AFSCs cultured in PRIME-XV AFSC Expansion Medium after six passages showed positive SOX2 staining. Nuclei were counterstained with DAPI.



Immunofluorescence analysis of human AFSCs cultured in PRIME-XV AFSC Expansion Medium after six passages showed positive NANOG staining. Nuclei were counterstained with DAPI .



Osteogenic differentiation test of human AFSCs cultured in PRIME-XV AFSC Expansion Medium after six passages. The cells showed positive OSTEOCALCIN immunostaining after culture in the osteogenic differentiation medium. Nuclei were counterstained with DAPI.