

AmcelGrow[®] Medium

Catalog No.	IS1001 100ml IS1002 500ml
Introduction	AmcelGrow [®] Medium is a complete medium and is specifically optimized for the primary culture, or culture of passaged human amniotic fluid cells, and generation of chorionic villi (CV) cells in open (5% CO ₂) systems. No addition of serum is required, and fetal karyotyping time is greatly reduced, compared with use of the conventional medium.
Format	This is a one bottle complete medium formulation which contains L-Glutamine and antibiotics.
Reconstitution	Thaw AmcelGrow [®] Medium at refrigerator temperatures (2-8°C) or by swirling bottle in a 37°C water bath. Always mix gently after thawing and before use.
Activity	AmcelGrow [®] Medium is tested for sterility, pH, osmolality, and endotoxin concentrations. In addition, each lot is tested for cell growth.
Use	For human amniotic fluid and chorionic villi samples.
Storage	Store at ≤ -18 °C. Protect from Light. After thawing, the medium should be stored at 2-8°C. The medium should be used within 7 days after thawing. Do not use if a visible precipitate is observed in the medium.
Expiry	Do not use beyond expiration date indicated on the product label.

Use of AmcelGrow[®] Medium does not guarantee the successful outcome of any prenatal analysis.

Protocol

In Vitro Culture of Amniotic Fluid Cells

1. Centrifuge 20ml of amniotic fluid at 750 rpm for 10 minutes.
2. Carefully decant the amniotic fluid from the cell pellet into a sterile test tube.
3. Resuspend the cell pellet with 2ml of amniotic fluid.
4. Add 2 ml of AmcelGrow® Medium (Catalog # IS1001/002) and gently swirl.
5. Culture 0.5ml of the cell suspension on each cover slip in the tissue culture dish. Be careful not to disperse the cells.
6. Place the dish in a 5% CO₂ incubator at 37 °C.
7. After 24 hours, add 1.5ml of AmcelGrow® Medium.
8. After 5 days, check the dish for presence of colonies. (Occasionally they will be visible only after 6-7 days.)
9. After the colonies first appear (5-7 days), replace the AmcelGrow® with fresh medium.
10. When the cultures have colonies of sufficient size (usually after 2 more days), proceed with harvesting.

Notes:

1. It is possible not to replace the AmcelGrow® Medium with fresh medium. In this case, the cells can be harvested after 9-11 days.
2. It is recommended to replace the AmcelGrow® with fresh medium for a second time (24 hours before harvesting.)

Flask Method Culture of Amniotic Fluid Cells – Open Systems

(Use the same procedure as for the above *in vitro* culture but with the following adaptations.)

1. Re-suspend the cell pellet with 4ml of amniotic fluid. Add 16ml of AmcelGrow® Medium and swirl gently.
2. Culture 5ml per each T25 tissue culture flask. Place cap loosely on the flask and incubate undisturbed at 37°C in 5% CO₂ atmosphere.
3. Check all flasks for growth after 5 days.

Chorionic Villi (CV) Testing

1. After obtaining the CV tissue, immediately place it in RPMI-1640 medium, supplemented with 1% heparin.
2. Transfer it to RPMI-1640 (without heparin) using a pasteur pipette.
3. Using sterile needles, separate the pieces of CV from the maternal tissue, and then store the CV in RPMI-1640.
4. Treat the CV tissue with Trypsin EDTA (0.25%) for 60 minutes at 37 °C.
5. Centrifuge, gently breakup, and treat the tissue pellet with collagenase Type V (100-120 units per ml) for 30 minutes at 37 °C.
6. Separate the cells mechanically using a pipette. Suspend the cells in AmcelGrow® and seed either in a flask (for biochemical analysis) or on plates (for genetic analysis).
7. **A. Plates:** After 2 days, add more AmcelGrow™ After another 1 day, replace all of the AmcelGrow® with fresh AmcelGrow® After an additional 5-6 days, harvest the cells.
B. Flasks: After 2 days, add an additional 2ml of AmcelGrow® After 5 more days, replace all the AmcelGrow® with fresh AmcelGrow®

Optimal dilution and reaction conditions to be determined by investigator usage.