

Product Information

AmnioPlus, Complete Medium for Cultivation of Amnion and Chorionic Villi Cells

Cat. No.: CY100-200 Volume: 100 ml

Product Description

AmnioPlus is a ready to use medium for in vitro diagnostic use. It has been specifically developed for the cultivation of human primary amnion and chorionic villi cells, which are intended for the preparation of karyograms, fluorescence in-situ hybridization and other cytogenetic methods.

The medium is supplied frozen and contains serum, L-glutamine and antibiotics.

Product Specifications

CO ₂ concentration, optimum	5.0 %
pH	6.9 - 7.1
Amnion Cell Growth	Positive
Chromosomal Aberrations	Negative
Chromosomal Morphology	Positive
Sterility	Tested
Storage	Store frozen at $\leq -15^{\circ}\text{C}$ in the dark until the expiry date given on the bottle label. After thawing, the medium should be stored at $+2^{\circ}\text{C}$ to $+8^{\circ}\text{C}$. The medium should be used within 2 weeks after thawing. Protect the medium from light.

Thawing

Thaw AmnioPlus Medium at $+37^{\circ}\text{C}$ in a water bath and mix gently during and after thawing to obtain a homogeneous medium. Note that the medium already contains L-glutamine and 50 $\mu\text{g/ml}$ gentamycin.

Protocol

The medium may be used in both open and closed culture systems.

Important information: Supplementation of AmnioPlus Medium is neither necessary nor recommended.

It is recommended to use cells from 2.5 ml of amniotic fluid per one coverslip. The following protocol and the volumes indicated are only general guidelines for use.

This high quality medium can be used within established procedures. It is up to the user to adopt either parts or all of the optimized protocol described below.

In Situ Culture of Amniotic Fluid Cells

1. Concentrate the cells by centrifugation of the amniotic fluid: Centrifuge 20 ml 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. Re-suspend the cell pellet with 2 ml of amniotic fluid.

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4. Add 2 ml of AmnioPlus Medium and swirl gently.
5. Culture 0.5 ml of the cell suspension on each coverslip in a tissue culture dish.
6. Incubate cultures at +37°C in a 5 % CO₂ atmosphere.
7. Add 2 ml of AmnioPlus Medium to each culture on day 2.
8. Check cultures for growth after 4 to 5 days. Feed cultures once growth has been observed. To feed cultures, carefully aspirate all of the exhausted culture medium and replace with 2 ml of fresh AmnioPlus Medium. Recommendation: feed cultures every 2 days.
9. When the cultures have colonies of sufficient size, proceed with harvesting.
10. For best results, feed cultures with AmnioPlus Medium the day before the harvest.

Flask Method Culture of Amniotic Fluid Cells

Use the same procedure as for the in situ culture, with the following adaptations:

1. Re-suspend the cell pellet with 4 ml of amniotic fluid. Add 16 ml of AmnioPlus Medium and swirl gently.
2. Culture 5 ml per T25 flask. Place the cap loosely on the flask and incubate undisturbed at +37°C in a 5 % CO₂ atmosphere.
3. Check all flasks for growth after 5 days.
4. For best results, feed cultures with AmnioPlus Medium the day before the harvest.

Recommendations for Closed Systems

AmnioPlus Medium may be used in closed culture systems as long as the physiological pH of 6.9 to 7.4 is maintained. Closed systems depend on adequate buffering capacity of media.

- Method 1: Supplement AmnioPlus Medium with 2 % (v/v) sterile 1.0 HEPES solution. The HEPES solution must be set to pH 7.0 at +20°C. HEPES supplemented medium can subsequently be used on cells in closed culturing flasks.
- Method 2: Pre-equilibrate the flask containing AmnioPlus Medium and cells at +37°C in a 5 % CO₂ atmosphere for 1 hour prior to closing the flask.
- Method 3: Flush each culture flask containing AmnioPlus Medium and cells with 5 % CO₂ – 95 % air through 0.2 µm sterile filter for 20 seconds. Tighten the caps and incubate the flasks at +37°C.

Precautions and Disclaimer

- For *in vitro* diagnostic use. The medium is not intended for therapeutic use.
- Do not use if a visible precipitate is observed in the medium.
- Use of AmnioPlus Medium does not guarantee the successful outcome of any prenatal diagnostic testing.
- Do not use AmnioPlus Medium beyond the expiration date indicated on the product label.