

UAB "LT Biotech"

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**AmnioFirst**  
**Complete Medium for Cultivation of Amnion and Chorionic Villi Cells**  
**Cat. No. : AF100ML (100 ml)**

**General Information**

AmnioFirst 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 preselected serum, L-glutamine and antibiotics.

|  |   |
|--|---|
| Appearance                             | Clear yellow to red frozen liquid   |
| CO <sub>2</sub> concentration, optimum | 5%  |
| Storage and shelf life                 | Store at ≤-15°C protected from light.<br>Once opened, store at +2°C to +8°C and use within 2 weeks. |
| Shipping conditions                    | Frozen (Dry ice)  |
| Thawing                                | +37°C in water bath and swirl gently to homogenize.   |

For lot specific data (**Certificate of Analysis**) please refer to our website: [www.capricorn-scientific.com/products/](http://www.capricorn-scientific.com/products/)

**Formulation**

The Formulation is based on the basal medium MEM Alpha Modification. Note that the liquid proprietary formulation of the medium already contains preselected fetal bovine serum, L-glutamine and 50 µg/ml gentamycin.

**Instructions for Use**

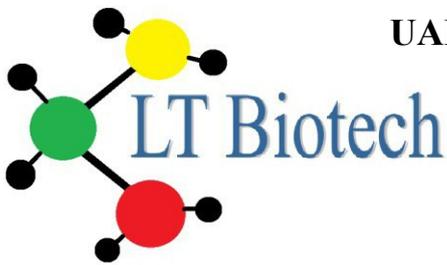
The medium may be used in both open and closed culture systems.

*Important information*

Supplementation of AmnioFirst 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. Resuspend the cell pellet with 2 ml of amniotic fluid.
4. Add 2 ml of AmnioFirst 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% CO<sub>2</sub> atmosphere.



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7. Add 2 ml of AmnioFirst 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 AmnioFirst 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 AmnioFirst Medium the day before the harvest.

### *Flask Method Culture of Amniotic Fluid Cells*

1. Use the same procedure as for the in situ culture, with the following adaptations:
2. Re-suspend the cell pellet with 4 ml of amniotic fluid. Add 16 ml of AmnioFirst Medium and swirl gently.
3. Culture 5 ml per T25 flask. Place the cap loosely on the flask and incubate undisturbed at +37°C in a 5% CO<sub>2</sub> atmosphere.
4. Check all flasks for growth after 5 days.
5. For best results, feed cultures with AmnioFirst Medium the day before the harvest.

### *Recommendations for Closed Systems*

AmnioFirst 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 AmnioFirst 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 AmnioFirst Medium and cells at +37°C in a 5% CO<sub>2</sub> atmosphere for 1 hour prior to closing the flask.

Method 3: Flush each culture flask containing AmnioFirst Medium and cells with 5% CO<sub>2</sub> – 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 AmnioFirst Medium does not guarantee the successful outcome of any prenatal diagnostic testing.

Do not use AmnioFirst Medium beyond the expiration date indicated on the product label.