**Product Description**

The *in vitro* cultivation of amniotic fluid cells and chorionic villi is an essential part of every diagnostic cytogenetics laboratory, since the preparation of metaphase chromosome spreads is dependent upon obtaining cells in division. Amniocentesis and chorionic villi sampling are the major invasive diagnostic procedures used for the detection of fetal chromosomal abnormalities. BIOAMF-3 is an improved medium, specifically optimized for the primary culture of human amniotic fluid cells and chorionic villi samples used in prenatal diagnostic testing. The medium is supplied frozen and contains serum, glutamine and antibiotics.

**Precaution 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 Biological Industries BIOAMF-3 Medium does not guarantee the successful outcome of any prenatal diagnostic testing.
- Do not use BIOAMF-3 Medium beyond the expiration date indicated on the product label.

**Storage and Stability**

BIOAMF-3 Medium should be kept frozen at -20°C. After thawing, the medium should be stored at 2-8°C. The medium should be used within 14 days after thawing. Protect the medium from light.

**Instructions for Use**

Thaw BIOAMF-3 Medium at refrigerator temperatures (2-8°C). Mix gently after thawing.

Note that the medium already contains L-Glutamine and antibiotics.
**Procedure**

BIOAMF-3 Medium may be used for:
- Primary culture of amniotic fluid cells
- Culture of passaged amniotic fluid cells
- Propagation of chorionic villus cells

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

It is recommended to use cells from 2.5ml of amniotic fluid per one coverslip.

The following protocol and the volumes indicated are only general guidelines for use.

**Flask Method Culture of Amniotic Fluid Cells – Open and Closed Systems**

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

1. Re-suspend the cell pellet with 4ml of amniotic fluid. Add 16ml of BIOAMF-3 Medium and swirl gently.
2. Culture 5ml per each T25 flask. Place the cap loosely on the flask and incubate undisturbed at 37°C in 5% CO₂ atmosphere.
   
   **For Closed Systems:** Flush each culture flask with 5% CO₂ – 95% air through 0.2µ sterile filter for 20 seconds. Tighten the caps and incubate the flasks at 37°C.
3. Check all flasks for growth after 5 days.

**Quality Control**

BIOAMF-3 Medium is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each batch is tested for cell growth using primary human amniotic fluid cells in a leading clinical cytogenetics laboratory.

**In Situ 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. Re-suspend the cell pellet with 2ml of amniotic fluid.
4. Add 2ml of BIOAMF-3 Medium and swirl gently.
5. Culture 0.5ml of the cell suspension on each coverslip in a tissue culture dish.
6. Incubate cultures at 37°C in 5% CO₂ atmosphere.
7. Flood cultures on day 2 with 1.5ml of BIOAMF-3 Medium.
8. After 5 days, check the cultures for the presence of colonies.
9. After the colonies first appear [5-7 days], replace the medium with fresh BIOAMF-3 Medium.
10. When the cultures have colonies of sufficient size, proceed with harvesting.

Note: It is recommended to replace the medium with fresh BIOAMF-3 Medium the day before harvesting.