



BIO-AMF[™]-2

Complete Medium for Human Amniotic Fluid and Chorionic Villi Samples

Cat. No.: 01-194-1
Store at: -20°C

Instructions for Use

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. BIO-AMF[™]-2 Medium is 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.
- Use of Biological Industries BIO-AMF[™]-2 Medium does not guarantee the successful outcome of any prenatal diagnostic testing.
- Do not use BIO-AMF[™]-2 Medium beyond the expiration date indicated on the product label.
- The product is prepared by an aseptic, strict, validated process in order to minimize as possible the risk for contamination and to ensure the safety and quality of the product. The membrane filtration and aseptic process is validated by media fill to ensure a safety level of 1 to 5000 bottles. Due to the residual risks of sample contamination, and the severity in such case, we recommend to establish parallel test systems as part of the procedure.

Storage and Stability

BIO-AMF[™]-2 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 7 days after thawing. Protect the medium from light.

Instructions for Use

Thaw BIO-AMF[™]-2 Medium at refrigerator temperatures (2-8°C). Mix gently after thawing.

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

Procedure

BIO-AMF™-2 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.

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 BIO-AMF™-2 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 BIO-AMF™-2 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 BIO-AMF™-2 Medium.
10. When the cultures have colonies of sufficient size, proceed with harvesting.

Note: It is recommended to replace the medium with fresh BIO-AMF™-2 Medium the day before harvesting.

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 BIO-AMF™-2 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

BIO-AMF™-2 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.

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