Product Information / Instruction for Use

AmnioPrime
Complete Medium for Cultivation of Amnion and Chorionic Villi Cells
Cat. No. APR-B (100 ml), APR-A (500 ml)

Intended Purpose

AmnioPrime cell culture medium supports the growth of human primary amniotic and chorionic villus cells intended for subsequent chromosome analysis (karyotyping, fluorescence *in situ* hybridization and other cytogenetic procedures).

General Information

AmnioPrime is a ready to use medium for *in vitro* diagnostic use and should only be administered by qualified professionals. 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. AmnioPrime is a non-automatic, *in vitro* Diagnostic class A sterile product intended for laboratory use.

Product Specifications

Appearance	Clear yellow to red frozen liquid
CO_2 concentration, optimum	5 %
Storage and shelf life	Store at ≤-15°C protected from light. Do not use this product after its expiry date. 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:
https://www.capricornscientific.com/en/services/certificate-of-analysis

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 gentamicin.

Instructions for Use

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

Important information:

Supplementation of AmnioPrime Medium is neither necessary nor recommended. It is recommended to use 0.5 ml of cell suspension 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 the optimized protocol described below either partially or completely.

***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.

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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 AmnioPrime 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_2 atmosphere.
7. Add 2 ml of AmnioPrime 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 AmnioPrime Medium.
9. Recommendation: feed cultures every 2 days.
10. When the cultures have colonies of sufficient size, proceed with harvesting.
11. For best results, feed cultures with AmnioPrime Medium the day before the harvest.

Harvesting of Amniotic Fluid Cells:

1. For harvesting, add 50 µL of Colcemid solution (Cat. No. COL-H) to each tissue coverslip and incubate for 20 minutes at 37°C in 5 % CO_2 atmosphere.
2. Carefully add 1 ml hypotonic solution, e.g., 0.075 M KCl (KCL-B).
3. Incubate at room temperature for 10-12 minutes and remove KCl solution.
4. Freshly add 2 ml KCl solution and incubate for 12 minutes at room temperature to facilitate swelling and bursting of cells.
5. Add 1 ml fresh, ice-cold fixative (6 methanol: 1 acetic acid) incubate for 12 min at room temperature and carefully remove supernatant from the tissue coverslip.
6. Repeat step 5 two to three times with 2 ml fresh, ice-cold fixative (3 methanol: 1 acetic acid) and do not remove the solution after the last cycle.
7. Gently remove the tissue coverslip from the dish using a forceps and allow excess liquid to drain by placing one edge of the coverslip onto a clean paper towel.
8. Create a humid environment by placing a 35 mm dish onto a clean wet paper towel. Then, gently lean the edge of the tissue cover slip against the 35 mm dish, cell side showing up, allowing the cover slip to dry gently for 2 to 3 min.
9. Label the back of each coverslip, then place for 4 to 24 h on a 60°C slide warmer, to allow samples to dry.
10. At this stage, the preparation can be stained with Orecin or Giemsa. For Giemsa staining, the most widely used method, you can use one of the common staining protocols for tissue coverslips or the protocol established in your laboratory.

Flask Method Culture of Amniotic Fluid Cells:

- Use the same procedure as for the in-situ culture, with the following adaptations:
- Resuspend the cell pellet with 4 ml of amniotic fluid. Add 16 ml of AmnioPrime Medium and swirl gently.
- Culture 5 ml per T25 flask. Place the cap loosely on the flask and incubate undisturbed at +37°C in a 5 % CO_2 atmosphere.
- Check all flasks for growth after 5 days.
- For best results, feed cultures with AmnioPrime Medium the day before the harvest.

Recommendations for Closed Systems:

AmnioPrime 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.

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Method 1:	Supplement AmnioPrime Medium with 2 % (v/v) sterile 1.0 M 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 AmnioPrime Medium and cells at +37°C in a 5 % CO_2 atmosphere for 1 hour prior to closing the flask.
Method 3:	Flush each culture flask containing AmnioPrime Medium and cells with 5 % CO_2 – 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 with humans or animals.
- Application only by qualified professionals.
- Maintaining the sterility of the product is necessary for its use and must be strictly observed by the user.
- Do not use AmnioPrime Medium if the packaging is damaged and thus sterility is impaired.
- Each laboratory is obliged to perform representative tests according to the valid legal regulations and in its own environment to ensure that it is suitable for this purpose before the medium can be used in routine diagnostics.
- The patient specimens are biological material and therefore safety precautions must be taken according to local regulations for working with potentially infectious material.
- Use of AmnioPrime Medium does not guarantee the successful outcome of any prenatal diagnostic testing.
- Do not use AmnioPrime Medium beyond the expiration date indicated on the product label.
- Frozen condition must be maintained until first use.
- Report serious incidents that have occurred in connection with this product to the manufacturer and the appropriate authorities.

Important Note

Occasionally, the formation of calcium oxalate crystals is possible, but these have not shown any negative influence on cell growth.

Signs and Symbols

REF	Catalog number
LOT	Batch Code
	Storage conditions: temperature limit
	Expiration date
STERILE A	Aseptic filling

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IVD	*In vitro* diagnostics
	Manufacturer
	Protect from sunlight
	Date of manufacture
	CE Marking
	See instruction manual
	Do not use if packaging is damaged

Manufacturer

Capricorn Scientific GmbH
Auf der Lette 13 A
35085 Ebsdorfergrund
Germany

Safe disposal

Waste treatment methods
Product
Dispose of in accordance with local regulations. Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

Environmental precautions
Do not let product enter drains.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).