
	Document ID:	TDS-AMG-001-100ML	Version:	002
	Date of Issue:	10-JUL-2023	Approved by:	Dr. Iman Kamranfar
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	Title:	TECHNICAL DATASHEET		

AMNIOGROW™

Complete Karyotyping Medium for Amnion and Chorionic Villi Cells

Filtration, Treatment	Sterile Filtered; Contains preselected FBS, L-Glutamine and gentamicin.
Product Code	AMG-001-100ML
Shelf Life	24 months from DOM
Storage Temperature	Store between -5°C to -20°C protected from light. Once opened, store at +2°C to +8°C and use within 2 weeks
Shipping Temperature	Frozen (Dry ice)
Thawing	+37°C in water bath and swirl gently to homogenize
CO2 concentration, optimum	5 %

QC Specifications

Physical and Chemical Analysis	Method	Specifications	Units
Appearance	Visual	Clear amber to red frozen liquid	n/a
pH at RT	Electronic pH Meter	6.8 - 7.6	n/a
Osmolality	Osmometer	Test and report	mOsm/kg
Endotoxin	LAL Kinetic	≤ 10.0	EU/ml
Sterility			
Aerobic Bacteria	EP 2.6.1	Not detected	n/a
Anaerobic Bacteria	EP 2.6.1	Not detected	n/a
Fungi (Yeast & Mold)	EP 2.6.1	Not detected	n/a
Mycoplasma	qPCR	Not detected	n/a

GENERAL INFORMATION/FORMULATION

This medium is ready to use product. 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.

The medium is formulated based on the basal medium MEM Alpha Modification and already supplemented with preselected foetal bovine serum, L-Glutamine and 50 µg/ml gentamicin.

INSTRUCTION FOR USE



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

Important information:

This medium is ready to use and no further supplements are needed. 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 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.
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 the medium and swirl gently.

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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 the 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 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 the 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. Resuspend the cell pellet with 4 ml of amniotic fluid. Add 16 ml of the 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 % CO₂ atmosphere.
4. Check all flasks for growth after 5 days.
5. For best results, feed cultures with this medium the day before the harvest.

Recommendations for Closed Systems:

This 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 this 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 this 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 this 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

The medium is not intended for therapeutic use.

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.

Do not use if a visible precipitate is observed in the medium.

Use of this medium does not guarantee the successful outcome of any prenatal diagnostic testing.

Do not use this medium beyond the expiration date indicated on the product label.