

AmnioMAX™-C100 and AmnioMAX™-II Complete Medium

Catalog Numbers 12558-011, 17001-082, 12556-015, 17001-074, 17001-094, 17001-095, 12556-023, 12556-096, 11269-016, 11269-097, A5557001

Pub. No. MAN0018473 Rev. E.0



WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](https://www.thermofisher.com/support).

Intended use

AmnioMAX™ products have been formulated and qualified for the in vitro propagation of primary cultures of human amniotic fluid cells and chorionic villus samples for use in prenatal diagnostic testing.

Cytogenetic products are for professional use. They are used in medical laboratories by personnel who have received specialized education and training with regard to procedures utilizing *In Vitro* Diagnostic (IVD) products. IVD products of this type are not intended as sole determinant in a diagnostic situation. Test results are interpreted by a healthcare professional as part of the clinical management of a patient.

Principle and explanation of procedure

Amniotic fluid provides a source of fetal material used in prenatal diagnostic testing. Amniotic fluid samples can be cultured as a source of metaphase cells for chromosome analysis and to provide additional material for biochemical and DNA-based testing

AmnioMAX™ products have been optimized to maximize colony attachment, growth rates, pH stability, and to provide prolific metaphasic yield.

- AmnioMAX™-C100 Complete Medium Kit contains an optimized basal medium and supplement containing Fetal Bovine Serum (FBS), gentamicin, and L-glutamine to maximize cell attachment and growth. This optimized medium also has an enhanced buffering system that provides greater pH stability during culture manipulations. AmnioMAX™-C100 Basal Medium requires supplementation with AmnioMAX™-C100 Supplement.
- AmnioMAX™-II Complete Medium is a second-generation formulation to improve cell morphology and provide cleaner cultures in a ready-to-use and convenient format which already contains antibiotics (gentamicin), L-glutamine, and FBS. AmnioMAX™-II Complete Medium is a nutritionally complete medium and requires no further supplementation.
- This product is sterile filtered.

Contents and storage

All quality control testing results are reported on lot-specific Certificate of Analysis available on our website: [thermofisher.com](https://www.thermofisher.com).

Product	Cat. No.	Storage	Shelf life ^[1]
AmnioMAX™-C100 Complete Medium Kit virtual, for ordering puposes only contains: - AmnioMAX™-C100 Basal Medium (1X) - AmnioMAX™-C100 Supplement	12558-011 17001-082 12556-015	—	—
AmnioMAX™-C100 Basal Medium (1X) 90 mL 450 mL 10 × 450 mL 20 × 90 mL	17001-082 17001-074^[2] 17001-094 17001-095	Protect from light 2°C to 8°C	16 months
AmnioMAX™-C100 Supplement 15 mL 75 mL 20 × 75 mL	12556-015 12556-023 12556-096	Protect from light –20°C to –5°C	16 months
AmnioMAX™-II Complete Medium 100 mL 100 mL (For China only) 20 × 100 mL	11269-016 A5557001^[3] 11269-097	Protect from light –20°C to –5°C	18 months

^[1] Shelf life is determined from Date of Manufacture. Do not use beyond the labelled expiration date.

^[2]  Dual manufactured.

^[3] Shipping condition: less than –5°C.

Related materials

Unless otherwise indicated, all materials are available through [thermofisher.com](https://www.thermofisher.com). "MLS" indicates that the material is available from [fisherscientific.com](https://www.fisherscientific.com) or another major laboratory supplier.

Item	Source
KaryoMAX™ Colcemid™ Solution, liquid (10 µg/mL), in HBSS	15210040
KaryoMAX™ Colcemid™ Solution, liquid (10 µg/mL), in PBS	15212012
KaryoMAX™ Giemsa Stain Stock Solution	10092013
Gurr Buffer tablets (pH 6.8)	10582013
Nunc™ 15 mL Conical Sterile Polypropylene Centrifuge Tubes	339651
Nunc™ 50 mL Conical Sterile Polypropylene Centrifuge Tubes	339653
Nunc™ EasYDish™ Dishes (35 mm)	150460
Nunc™ Thermanox™ Coverslips (25 mm)	174985
Nunc™ Serological Pipettes (5 mL)	170355
HBSS, calcium, magnesium, no phenol red	14025092
PBS	10010023

Precautions

Do not use the product if packaging, including bottles and vials, have been compromised and/or show evidence of microbial contamination, cloudy appearance, discoloration, drying, cracking, or other signs of deterioration. AmnioMAX™ -C100 and AmnioMAX™-II Complete Medium should be received frozen; therefore, a thawed product is an indication of a compromised product.



CAUTION! Human amniotic fluid is biohazardous. Follow standard precautions for handling, storage and disposal.



CAUTION! Do not use for injection or infusion! Please report any serious incidents in relation to the device to the manufacturer and the Competent Authority of the EU Member State in which the user and/or patient is established.

Precautions

Do not use the product if packaging, including bottles and vials, have been compromised and/or show evidence of microbial contamination, cloudy appearance, discoloration, drying, cracking, or other signs of deterioration. PB-MAX™ Karyotyping Medium should be received frozen; therefore, a thawed product is an indication of a compromised product.



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Procedural guidelines

- Always use proper aseptic techniques and work inside a laminar flow hood. Consult our [Gibco Cell Culture Basics](#) for aseptic handling.
- Perform all incubations in a humidified 37°C, 5% CO₂ incubator unless otherwise specified.

Guidelines for AmnioMAX™-C100 and AmnioMAX™-II Complete Medium

- AmnioMAX™-C100 and AmnioMAX™-II Complete Medium is supplied frozen, ready to use upon thawing.
- Thaw at 2–8°C, then mix by gently swirling to ensure homogeneity. Do not thaw at 37°C. This may result in formation of a precipitate and should be avoided.
- AmnioMAX™ Media contain Fetal Bovine Serum (FBS); flocculent debris can develop upon thawing and storage.
- Thawed, unopened AmnioMAX™-C100 and AmnioMAX™-II Complete Medium can be stored protected from light at 2–8°C for up to two months within the labeled expiration date.
- Once opened, use AmnioMAX™-C100 and AmnioMAX™-II Complete Medium products within 10 days for maximal growth performance.
- Avoid repeated warming/cooling and prolonged exposure to light.
- Do not use beyond the labeled expiration date.

Before you begin

1. Aseptically add the entire contents (15 mL) of AmnioMAX™-C100 Supplement to 90 mL of AmnioMAX™-C100 Basal Medium.
2. Mix by gently swirling to ensure homogeneity.
3. Store protected from light at 2–8°C until use.

Additional supplementation to AmnioMAX™ products is not recommended. Addition of Fungizone™ can be toxic.

Prepare amniotic fluid samples for culturing

1. Obtain amniotic fluid in 20-mL syringes or tubes that have been approved for cell culture, then maintain samples at room temperature until they are processed.
2. Gently invert the original container of amniotic fluid sample to resuspend cells.
3. Transfer samples to conical tubes (Cat. No. 339651 or 339653), then centrifuge at 100 × *g* at room temperature.
4. Label 35-mm Nunc™ EasYDish™ Dishes (Cat. No. 150460) on both the top and bottom. Using forceps, place sterile Nunc™ Thermanox™ coverslips (Cat. No. 174985) in the bottom of the dishes.
5. Taking care not to disturb the cell pellets, remove the conical tubes from the centrifuge. In the biosafety cabinet, remove the supernatant from each tube. Leave ~1 mL of fluid above the pellet.
6. Transfer the supernatant to another tube to save for future biochemical testing.
7. Carefully aspirate any remaining supernatant above each pellet, then add 0.5 mL of AmnioMAX™-II Complete Medium per culture to be seeded from the pellet.
8. Tap gently on the side of the tube to resuspend the pellet, then distribute the cell suspension equally to the center of the coverslip.
Be careful to confine the suspension to the coverslip.
9. Incubate cultures for 24 to 48 hours in a humidified incubator at 37°C and 5% CO₂.

Flood the cultures with AmnioMAX™-II Complete Medium

1. Gently add 2 mL of pre-warmed AmnioMAX™-II Complete Medium to the culture dishes.
2. Incubate the cultures for 2 days in a humidified incubator at 37°C and 5% CO₂.

Feed cultures

1. Under the hood, use a sterile 5-mL serological pipette to remove the medium from the periphery of the dishes so that the cells are not disturbed.
2. Add 2 mL of pre-warmed AmnioMAX™-II Complete Medium, then incubate the cultures in a humidified incubator at 37°C and 5% CO₂ for 3 days.

Harvest cells for metaphase chromosome preparation

1. Starting on the fifth day in culture, periodically check for colony formation and cell growth using an inverted microscope.

Medium should be changed every 48 to 72 hours until colonies are observed.
2. When colony formation is apparent, add 50 µL of 10 mg/mL KaryoMAX™ Colcemid™ Solution (Cat. No. 15210040 or Cat. No. 15212012) to each 2-mL coverslip culture, then gently rotate the dish to mix.
3. Incubate for 20 minutes in a humidified incubator at 37°C and 5% CO₂.
4. Remove the dishes from the incubator, then gently add ~1 mL of hypotonic solution (HBSS Cat. No. 14025092 or PBS Cat. No. 10010023) around the inner edge of dish.
5. Let the dish stand for 10–12 minutes. Aspirate the hypotonic solution/medium mix from around the edge of the dish.
6. Add 2 mL of hypotonic solution, then let the dish stand for 12 minutes.
7. Gently add ~1 mL of fresh 6:1 methanol/glacial acetic acid fixative, then let the dish stand for 12 minutes. Aspirate the fixative solution from around the edge of the dish.
8. Repeat step 7 with fresh 3:1 fixative two or three times for 10 minutes each time.

Note: Do not remove the final fixative.
9. Lift the coverslip out of the dish with fine forceps, then place the edge of the coverslip on a paper towel to drain excess fixative.
10. Place a 35-mm dish on top of wet paper towels (to create a humid environment for the drying process), then lean the corner of the coverslip (cell side up) against the cover. Allow to dry for 2–3 minutes.
11. Move the 35-mm dish cover to a 60°C slide warmer, then lean the coverslip (cell side up) against the cover. Allow to dry for ~10 minutes.
12. Gently label the back of each coverslip with a Gram stain pen, then leave the coverslips cell side up on the 60°C slide warmer for at least 4 hours, but not more than 24 hours.
13. Stain the coverslips using standard recipes and times for staining slides.
14. After the coverslips are stained and dry, analyze the metaphase chromosomes, then interpret the results.

Stain with Giemsa Stain

Banding of chromosome with enzymes and stains is essential to identifying normal and abnormal chromosome structures.

1. Prepare six Coplin jars according to the following table:

Jar number	Contents
1	0.125% trypsin/0.9% NaCl mixture
2	0.9% NaCl for rinsing
3	0.9% NaCl for rinsing
4	Gurr Giemsa stain (R66) mixed with Gurr 6.8 buffer and acetone
5	Gurr 6.8 buffer for rinsing
6	Gurr 6.8 buffer for rinsing

2. Place a slide for a prescribed amount of time in the jar containing the trypsin/NaCl mixture (Jar 1).

This time can be as short as 10 seconds or as long as 2 minutes, depending on the activity level of the trypsin being used.
3. After the trypsin time has elapsed, remove the slide, then rinse by sequential dipping into the 0.9% NaCl rinsing jars (Jars 2 and 3).
4. Place the slide in the staining jar (Jar 4) containing the Gurr stain and buffer for 5 minutes.

This time can vary depending on the strength of the stain used.
5. Remove the slide from the jar, then rinse by sequential dipping into the two Gurr buffer rinsing jars (Jars 5 and 6).
6. Remove the slide from the last rinse to air dry, then coverslip the slide with Cytoseal™ 60.

It is allowed to dry in the oven (50°C) after which it is ready for metaphase scanning under the microscope.

Quality assurance/control

Every lot of AmnioMAX™ -II Complete Medium and AmnioMAX™ -C100 Complete Medium is performance tested by a certified US reference cytogenetics laboratory to ensure consistently superior performance. Pooled primary human amniotic fluid samples are cultured for six days in AmnioMAX™ -II Complete Medium or AmnioMAX™ -C100 Complete Medium before measuring the total number of colonies and total number of mitotic colonies. In addition, each lot is tested for pH and osmolality, and must pass a sterility test prior to lot release.

Label symbols

The symbols present on the IFU and labels that are not globally recognized as per ISO 15223 are explained in the following table.

Read SDS	READ SAFETY DATA SHEET Consult Safety Data Sheet for risks associated with product.
UKRP	AUTHORISED REPRESENTATIVE IN THE UNITED KINGDOM

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms

and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

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Revision history: Pub. No. MAN0018473

Revision	Date	Description
E.0	26 January 2023	Updated manufacturing address to Paisley. Removed UKCA symbol. Minor edits.
D.0	29 November 2022	Footnote added to SKU A5557001.
C.0	29 July 2022	Catalog numbers were added and minor corrections were made to the product information sheet.
B.0	4 March 2020	- Updated EC Rep address to The Netherlands - Added manufacturing location into Contents and storage table
A.0	15 March 2019	Initial release.

The customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.

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