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Amniochrome™ Media

Product Information

Amniochrome™ Plus	02-026E	100 ml
	02-026F	500 ml
Amniochrome™ Pro		
	02-035E	100 ml
	02-035F	500 ml
Amniochrome™ II	**Modified Complete Media System**	
	12-756EZM	100 ml
	(Supplied as: 12-756EM+17-524ZM)	
	12-756FCM	500 ml
	(Supplied as: 12-756FM+17-524CM)	

CAUTION

Handle in accordance with established bio-safety practices.

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or its subsidiaries.

Product Feature Summary

Intended Use

Amniochrome™ Media are intended for use *in vitro diagnostic* procedures and have been designed for establishing primary cultures of human amniotic fluid cells (AFC) and chorionic villus samples (CVS), which may then be used in karyotyping, fluorescence *in-situ* hybridisation (FISH) and other cytogenetic procedures. The products have been rigorously quality control tested by a leading European clinical cytogenetic diagnostic reference laboratory for this application.

Background

Since the first successful culture of Amniotic fluid cells in the 1960s amniocentesis and chorionic villus sampling have become the most common used invasive diagnostic tests in prenatal diagnostic.

Amniotic fluid cells, which represent a variety of histotypes[1-3], reflect the foetal status concerning their biochemical and cytogenetic composition. Therefore these cells can be used in clinical diagnosis for DNA-analysis[4] and to monitor foetal chromosome abnormalities[5-6] and defects in metabolic enzymes[7-10].

For successful prenatal diagnostic a rapid *ex vivo* cultivation and karyotyping of these amniotic fluid cells is needed.

Amniotic fluid cells and chorionic villi cells can be propagated in either conventional cell culture medium supplemented with bovine serum or in specialized culture medium[11-13].

Product Description

Amniochrome™ Media were developed specifically for the *in vitro* prenatal diagnostic testing of human amniotic fluid and CVS specimens and designed for ease of handling. Each formulation have been optimised by thorough performance testing on primary human amniotic fluid and chorionic villus biopsy samples with attachment and growth promoting substances to minimize diagnostic turn-around time by maximizing colony attachment and growth. Every manufactured lot of product is similarly tested against rigorous standards to ensure necessary clinical performance.

Amniochrome™ Plus Medium

Amniochrome™ Plus Medium (02-026) is supplied frozen, ready-to-use and already contain antibiotics, L-glutamine and FBS, hormone and growth factors offering additional convenience to the end-user.
Amniochrome™ Plus is buffered with Sodium Bicarbonate and Phenol Red is present as a pH indicator. The complete formulation reduces the chance of technical error and culture contamination. In addition, this product supports more efficient cell attachment and cell growth resulting in early chromosome analysis.
Amniochrome™ Plus is offered in 100 ml and 500 ml configurations

Amniochrome™ Pro Medium

Amniochrome™ Pro Medium (02-035) is supplied frozen, ready-to-use and already contain antibiotics, L-glutamine and FBS, hormone and growth factors offering additional convenience to the end-user.
Amniochrome™ Pro is buffered with Sodium Bicarbonate and Phenol Red is present as a pH indicator. The complete formulation reduces the chance of technical error and culture contamination. In addition, this product supports more efficient cell attachment and cell growth resulting in early chromosome analysis.
Amniochrome™ Pro is offered in 100 ml and 500 ml configurations

Amniochrome™ II Modified Complete Medium System

Amniochrome™ II Modified consists of a refrigerated basal medium (cat. no. 12-756M) offered in 100 ml and 500 ml bottles, and a frozen supplement (cat. no. 17-524M) offered in a 7 ml and 35 ml configuration. The complete media system consisting in basal medium and supplement is supplied ordering 12-756EZM (100 ml) or 12-756FCM (500 ml).
The final medium already contains antibiotics, L-glutamine and FBS, hormone and growth factors offering additional convenience to the end-user.
Amniochrome II Modified is buffered with Sodium Bicarbonate and HEPES. Phenol Red is present as a pH Indicator.

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Storage Conditions and Shelf-life

Amniochrome™ Plus and **Amniochrome™ Pro**
Store at < -18°C and in the dark until the stated expiry date shown on the label.

Amniochrome™ II Modified Complete Media System
Store frozen supplement at < -18°C, in the dark. Store liquid basal medium at 2°C to 8°C in the dark until the stated expiry date shown on the label.

Limitation: Do not use beyond labelled expiration date.

Instruction for Use

Media Preparation

Amniochrome™ Plus and Amniochrome™ Pro Medium - should be thawed in a 30°C to 37°C water bath or incubator. Excessive temperature will degrade heat labile nutrients. If using a water bath, prevent the bottle caps from being completely submerged.
Gently swirl until the medium is completely thawed.
Proper periodic agitation is the key to prevent cryoprecipitate.

Amniochrome™ II Modified – Completely thaw supplement in a 30°C to 37°C water bath or incubator. Excessive temperature will degrade heat labile nutrients. If using a water bath, prevent the bottle caps from being completely submerged. Gently swirl until the medium is completely thawed.
Proper periodic agitation is the key to prevent cryoprecipitate.
Aseptically add the entire content of Amniochrome™ II modified supplement to a bottle of Amniochrome™ II basal medium, i.e. 7 ml of supplement to 100 ml basal or 35 ml of supplement to 500 ml basal.
Gently swirl resulting Amniochrome™ II modified complete medium to ensure thorough mixing. Avoid foaming.

Amniochrome™ Media shall be stored once opened in the dark at 2°C to 8°C and use within 2 weeks for maximal growth performance. Repeated warming / cooling and prolonged exposure to light should be avoided.

Amniochrome™ Media contain Foetal Bovine Serum (FBS); therefore flocculent debris may develop upon thawing and storage.

Antibiotics

Amniochrome™ Media contain Gentamycin, which is less inhibitive to growth compared to Penicillin and Streptomycin.

Performance Testing

Amniochrome™ Media are tested for sterility according to EP and analysed for pH, and endotoxin content. In addition to these standard specifications, each manufactured lot is tested for cell growth by an independent European Cytogenetics laboratory and the product performance is compared to a reference standard. A Certificate of Analysis (CoA) is available upon request.

Precautions

Please contact directly the Sales & Marketing Department at Lonza for any concerns relating to the product or ask your local distributor to do it on your behalf.

Do not use product if:

- Packaging appears compromised.
- Product appears cloudy or a visible precipitate is observed.
- Colour is not an orangish-red colour.

If product is received thawed or partially thawed, freeze immediately at < -18°C and contact Lonza.

FOR USE IN *IN VITRO* DIAGNOSTIC PROCEDURES REQUIRING THE CULTIVATION AND GROWTH OF HUMAN AMNIOTIC FLUID CELLS AND / OR CHORIONIC VILLUS BIOPSY SAMPLES (CVS) ONLY.

Additional supplementation to Amniochrome™ Complete Media is not recommended. Adding components or diluting the medium may result in negative effects on cell growth or chromosome integrity.

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Limitations

Each laboratory must carry out their own testing procedures on new media prior to releasing them to the lab for routine *in vitro* applications. Lonza contribution to these procedures is simply to provide a culture or handling medium and therefore Lonza do not guarantee the successful outcome of any testing based only on the use of Lonza medium.

Lonza recommends testing the patient samples in duplicate, using a cell culture medium previously released.

Each manufactured lot of **Amniochrome™ Media** are thoroughly performance tested on primary amniotic fluid isolates to ensure product performance for *in vitro* diagnostic use for this application.
Lonza cell culture liquid products are prepared by an aseptic process for which each step has been validated to ensure that all products meet the industry standard sterility assurance level of 10^{-3} i.e., product that demonstrates a contamination level of no more than 1 of 1000 units during the manufacturing process. The highest level of sterility assurance (equal to or greater than 10^{-6}) cannot be achieved without terminal sterilization, which is harmful to the performance of these cell culture products.

Cell Culture Protocols

The protocols below provide a guide for AFC and CVS culture using ***Amniochrome™ Media***. They can be used to replace either part of or all of existing optimised protocols for AFC and CVS cultures at the user's discretion. The majority of cytogenetic laboratories have their own protocols and ***Amniochrome™ Media*** can, in most cases, be simply substituted in current cell culture protocol. The most common culturing method uses an "open" system.

Open System / Closed System

Definition of "open" system: cultures growing in dishes with vented lids or in flasks/tubes with loosened caps in a 5% CO_2 atmosphere (gas incubator), which allows gaseous exchange.

Definition of "closed" system: cultures growing in a standard un-gassed, dry incubator in tightly sealed culture vessels.

Recommendations for Use of "Open" System

In situ method

1. Concentrate the cells by centrifugation of the amniotic fluid at low speed.
2. Remove 90-95% of the supernatant and resuspend the cell pellet in the remaining volume of the patient's own amniotic fluid. Dilute the concentrated cell suspension with sufficient Amniochrome™ Complete Medium to allow for final plating volume of 0.5 ml per coverslip (total of 4 coverslips) or 2 ml per flaskette.
3. Incubate cultures at 37°C in a 5% CO_2 atmosphere.
4. Add 2 ml of Amniochrome™ Complete Medium to each culture on day 2.
5. 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 Amniochrome™ Complete Medium. Recommendation: feed cultures every 2 days.
6. Check cultures for growth on/or after day 5, and harvest when sufficient colonies are observed.
7. For best results, feed cultures with Amniochrome™ Complete Medium the day before the harvest.

Flask method

1. Concentrate the cells by centrifugation of the amniotic fluid at low speed.
2. Remove 90-95% of the supernatant and resuspend the cell pellet in the remaining volume of the patient's own amniotic fluid. Dilute the concentrated cell suspension with sufficient Amniochrome™ Complete Medium to allow for final plating volume of 0.5 ml per coverslip (total of 4 coverslips) or 2 ml per flaskette.
3. Incubate cultures at 37°C in a 5% CO_2 atmosphere.
4. On day 5 check for growth. Remove medium and replace with fresh Amniochrome™ Complete Medium and harvest if sufficient cell growth is observed.

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5. Check culture for growth and completely change medium every other day thereafter until sufficient colonies are observed and are ready to harvest.
6. For best results, feed cultures with Amniochrome™ Complete Medium the day before the harvest.

Recommendations for Use of "Closed" Systems

Amniochrome™ Media can be used to culture cells in a "closed" system as long as the pH remains physiologic (pH = 6.9 to 7.5). Closed systems rely on the intrinsic buffering capacity of the medium in the absence of the benefit provided by the equilibrium between the bicarbonate in the medium and the 5% CO_2 present in an open system incubator. Closed systems work best in cloning applications with low cell density since higher cell densities produce acidic metabolites that can acidify the medium beyond its physiologic buffering capacity. ***Amniochrome™ Media*** is supplied at the correct pH; however occasionally the pH may fluctuate and need to be re-equilibrated. The maintenance of pH can be accomplished in the closed system by one of the 3 following methods:

- Method 1: Supplement ***Amniochrome™ Plus or Amnio-chrome™ Pro Medium*** with 2% (v/v) of sterile 1.0 M HEPES stock solution. The sterile 1.0 M HEPES must be adjusted to pH 7.0 at 20°C with 1.0 M NaOH. The HEPES supplemented medium is then combined with cells and incubated at 37°C with the culture flask closed.
- Method 2: Pre-equilibrate culture flasks containing ***Amniochrome™ Plus or Amniochrome™ Pro Medium*** and cells in an open 5% CO_2 incubator for one hour prior to closing the cap and culturing at 37°C.
- Method 3: Purge each individual culture flask containing ***Amnio-chrome™ Plus or Amniochrome™ Pro Medium*** and cells with 5% CO_2 - 95% air from a sterile pipette for 20 seconds. Then close the cap and culture in a closed system at 37°C. (It is recommended that a sterile plugged Pasteur pipette be attached to the CO_2 source to ensure sterility of the incoming gas).

- **Amniochrome II Modified Complete Medium** can be directly used in a closed system as it does contain HEPES to ensure appropriate pH maintenance**.**

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Ordering Information

Cat.No.	*Size*

Amniochrome™ Plus
Complete Ready To Use Medium for the Primary Culture of Amniotic Fluid and Chorionic Villi Cells Used in Cytogenetic Applications. For *In Vitro* Diagnostic Use.
Storage conditions: < -18°C

02-026E	100 ml
02-026F	500 ml

Amniochrome™ Pro
Complete Ready To Use Medium for the Primary Culture of Amniotic Fluid and Chorionic Villi Cells Used in Cytogenetic Applications. Optimized for growth rate. For *In Vitro* Diagnostic Use.
Storage conditions: < -18°C

02-035E	100 ml
02-035F	500 ml

Amniochrome™ II Modified Complete Medium System
Complete Medium for the Primary Culture of Amniotic Fluid and Chorionic Villi Cells Used in Cytogenetic Applications. For *In Vitro* Diagnostic Use.
Storage conditions: < -18°C (7 ml or 35 ml supplement)
2°C – 8°C (100 ml or 500 ml basal)

12-756EZM Amniochrome™ II Modified Complete Medium System (107 ml)
Supplied as:

12-756EM	Amniochrome™ II Basal Medium	100 ml
17-524ZM	Amniochrome™ II Modified Supplement	7 ml

12-756FCM Amniochrome™ II Modified Complete Medium System (535 ml)
Supplied as:

12-756FM	Amniochrome™ II Basal Medium	500 ml
17-524CM	Amniochrome™ II Modified Supplement	35 ml

Amniochrome™ is a trademark of CBM Intellectual Properties Inc.

For further information on this or other Lonza products, contact Technical Services at the following phone number: +32 87 32 16 11
E-Mail: techsup.europe@Lonza.com

You may also contact your Lonza Sales Representative or our World Wide Web site at www.Lonza.com

For *In Vitro* Diagnostic Use.

Not to drink.
Not to inject to human or animal.

CAUTION: Not for human or animal therapeutic use. Uses other than the labeled intended use may be a violation of local law.

MANUFACTURER

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The following chart displays the symbols with their definitions.

Symbol	Used for	Symbol	Used for
	Catalog number		*In vitro* diagnostic medical device
	Batch code		Sterilized using aseptic processing technique
	Used by YYYY-MM		Manufacturer
	Upper limit of temperature		Serial number
	Temperature limitation		Keep in the dark

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