Datasheet

Amniopan

Complete Medium for the Cultivation and Cytogenetic Analysis of Human Fetal Cells from Amniotic Fluid and Chorion Villi Biopsy Samples

Product	Description	Catalogue-No.	Size
Amniopan	**Complete medium for the cultivation and cytogenetic analysis of human fetal cells from amniotic fluid and chorion villi biopsy samples**	**P04-70100** **P04-70500**	**100 ml** **500 ml**

Product Description

Amniopan has been specifically developed for prenatal in vitro diagnostic use with human fetal cells from amniotic fluid or chorion villi biopsy (CVS) material for a standardized application. The media formulation was optimized on human fetal cells from amniotic fluid and CVS, with special emphasis on fast attachment of cells to the cell culture substrate and efficient cell growth to facilitate rapid diagnostic findings.

General Information

Prenatal diagnostic requires and crucially depends on various invasive and non-invasive techniques in order to examine the fetal nesting and to determine possible fetal genetic abnormalities or malfunctions. Amniocentesis and chorionic biopsy represent essential methods for the invasive diagnostic within the scope of a clinical examination of chromosome abnormalities of a fetus in order to assess the risk of genetic disorders.

Amniocentesis was established in the 1960s and has since then become a common procedure in the cytogenetic routine; cells cultured from these samples supply the source material for prenatal cytogenetic examinations and chromosomal analysis. A successful prenatal diagnostic procedure requires a rapid *in vitro* expansion of these cells. Traditionally, cell culture media containing fetal bovine sera (FBS) have been used. Besides these conventional media supplemented with FBS, more specialized cell culture media can be applied.

Amniopan is specifically designed for prenatal diagnostic testing and optimized for establishing primary cultures of human amniotic fluid cells (AFC) and chorion villi samples (CVS) which will be used for karyotyping, fluorescent in situ hybridization (FISH) and other cytogenetic methods. Before release, the product is tested for sterility and performance is monitored in a quality control test.

Storage conditions

Storage:	-20°C in the dark
Stability:	2 years
Size:	100 ml, 500 ml, other sizes on request

Composition

Amniopan is supplied frozen as a complete medium, ready-to-use in a 100 ml or 500 ml format. It is based on alpha-MEM and contains antibiotics, L-glutamine, Fetal Bovine Serum (FBS), hormones and growth factors. This product is manufactured under strictly supervised quality system in conformance with the ISO 9001/EN and ISO 13495 (medical products and in vitro diagnostics) requirements.

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Suitability

Amniopan is a highly efficient complete medium (ready-to-use) for cytogenetic analysis of human fetal cells from amniotic fluid and chorion villi biopsy samples.
FOR RESEARCH AND IN VITRO DIAGNOSTIC USE ONLY!
Not approved for human or animal therapeutic procedures.

Instructions for Use

Amniopan is supplied ready-to-use. No further supplementation is necessary, as antibiotics, L-glutamine and fetal bovine serum (FBS), hormones and growth factors are already included. Amniopan is buffered with NaHCO3 and phenol red is present as pH indicator. The formulation which is ready for use reduces the risk of technical errors and a contamination of the cultures.
Amniopan is a complete medium, supplied in sterile, frozen condition. Additional filtration is not recommended. Thawed Amniopan can be used up to 2 weeks when stored at 2-8°C. Repeated warming and cooling and exposure to light has to be avoided. I

- Thawing

Amniopan is ready-to-use and can be applied immediately after thawing in a water bath at 37°C, gently swirling the flask during the thawing procedure to achieve a homogenous mixture and to avoid a formation of precipitate. Alternatively, Amniopan can be thawed in an incubator with CO_2 – fumigation; a slight opening of the screw cap (1/4 turn) is recommended in order to allow for equilibration of the pH.

- Control of pH

Amniopan is adjusted to a correct pH value of 6.9 to 7.1 when it is supplied. Due to transport on dry ice or storage for prolonged time, a deviation of the initially adjusted pH value may occur. In this case, the following instructions will help to re-adjust the pH of Amniopan.

Amniopan contains phenol red as pH-indicator: Dark red indicates a high pH-value (basic). Phenol red turns dark red at pH 7.4. The medium is too basic and the pH can be corrected by equilibration. The screw cap should be opened slightly (1/4 turn) and the bottle should be placed in an atmosphere of 5% CO_2 (e.g. cell culture incubator) for 1 hour. A yellow color indicates a pH value which is too low. Phenol red turns yellow at pH 6.7. Yellow medium is too acidic and can be adjusted as described above for too basic Amniopan. Alternatively, the culture bottle can be fumigated. In this case a 5 % CO_2 – fumigation under sterile conditions is necessary. The color can be optically compared with a fresh, newly defrosted bottle in order to determine the correct color.

- Cell Culture Methods

In the following chapter you will find some recommendations for AFC and CVS cultures when using Amniopan medium. These methods may be replaced by others, modified or partially exchanged, depending on the availability of optimized or other procedures. The majorities of cytogenetic laboratories have their own methods and use the cell culture medium within the scope of these methods and conditions.

Most common cell culture methods are based on a so-called open system.

Open Systems versus Closed System

Definition open system: Cultures grow in plates with ventilated covers or in flasks with loosened or vented caps in 5% CO_2 atmosphere (incubator with fumigation) to allow gas exchange.

Definition closed system: Cultures grow in a sealed system in a standard dry incubator without fumigation by air – CO_2 mixture. In this case a suitable buffering of the culture medium has to be provided.

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- Recommendations for use in an open system:

1. Concentrate the cells of the amniotic fluid by centrifugation at low speed (e.g. 120-150 g).
2. Carefully remove 90-95% supernatant and re-suspend the pellet in the remaining volume of amniotic fluid. Dilute the cell suspension with a volume of Amniopan to achieve a plating volume of 0.5 ml per coverslip (total 4 coverslips) or 2 ml per flask.
3. Incubate the cultures at 37 °C in 5% CO_2 atmosphere.
4. Add 2 ml Amniopan to each culture on day two.
5. After 4 to 5 days check the cultures for growth. Feed the cultures when cell growth has been detected. For feeding, carefully aspirate the spent medium and replace by 2 ml fresh Amniopan medium. It is recommended to repeat feeding every two days.
6. As soon as a sufficient number of colonies can be observed (about day 5 onwards) harvest the cultures.
7. In order to achieve best results, feed cultures with Amniopan on the day before harvest. This procedure sets off a wave of division and produces plenty of mitoses.

- Recommendations for use in a closed system:

Amniopan medium may be used in sealed systems as long as the pH value is maintained at physiologic level (pH = 6.9 to 7.4). Sealed systems are dependent on an adequate buffering capacity of the medium. A closed system works best in cloning procedures with a low cell number as higher cell densities produce more acid metabolites which can bring down the pH value in the medium into an unphysiologic range.

- Suggestions for maintaining cultures at physiologic pH in a closed system:

Method 1: Add 2% (v/v) of sterile 1.0 M HEPES stock solution to Amniopan. The HEPES solution has to be adjusted to pH 7.0 at 20°C with sterile 1.0 M NaOH. The cells are added to the HEPES-supplemented medium and incubated in a sealed culture flask at 37°C.

Method 2: Pre-equilibrate the flask with Amniopan and cells in a 5% CO_2 incubator for 1 hour before sealing and culturing at 37°C.

Method 3: Each individual flask with Amniopan and cells is purged with 5% CO_2-95% air mixture by means of a sterile pipette (sterile filter interposed) for 20-30 sec. Close the system and incubate at 37°C.

- Troubleshooting for impaired cell growth

Impaired growth of cells could depend on several factors:
Number of cells too low
Volume of amniotic fluid to low
Time lapse between sampling and cell cultivation is too long
Poor quality of cell cultures with contamination by cellular debris or erythrocytes.

If the amniotic sample is already visibly bloody, the high number of erythrocytes can considerably disturb cell growth. In case of impaired cell growth due to a contamination with erythrocytes the following procedure has proved successful: complete change of medium when feeding the first time, and a complete change of medium including washing with DPBS (without Mg2+/Ca2+) when feeding the second time.

Precautions

Do not use product when the packaging appears compromised or visible precipitate is observed or the product is received thawed or partially thawed. Avoid prolonged exposure to light. As Amniopan contains FBS, flocculent debris may develop upon thawing and storage.

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Limitations

Each batch of Amniopan is thoroughly tested for biological performance to ensure cell growth and in vitro diagnostic use.

However, for in vitro diagnostic applications each laboratory should establish and regularly perform internal quality testing procedures when selecting new cell culture media or utilising new batches of media prior to releasing these to the clinical routine.
In particular, the contribution of PAN Biotech to these procedures is limited merely to providing a culture medium which has been tested and found suitable for the intended use. PAN Biotech therefore does not guarantee a successful implementation for specific settings especially in diagnostic procedures.
In addition, PAN Biotech can not be held responsible for damage due to absence of cell growth or diagnostic failure based solely on the use of PAN Biotech medium.

Only for in vitro diagnostic use by means of culture and growth of human fetal cells from amniotic fluid or chorion villi biopsy sample material. Not intended for human or animal therapeutic use.

Technical Support

In any case of questions or uncertainties please contact the customer service of PAN Biotech GmbH or the authorized local representative.
For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email (info@pan-biotech.com) or phone +49-8543-601630.

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