

AMNIO PAN

Directions for Use

Warning Information

Use biological material with respective safety precautions.

Product Properties

Application

AMNIOPAN is designed for the application in In-Vitro Diagnostic tests and optimized for established primary cultures of human amniotic cells and chorion villi biopsy samples which shall be used with the object of karyotyping, fluorescent in situ hybridization (FISH) and other cytogenetic methods. Before the lot release the product is tested for sterility and the content is chemically analyzed and tested in a rigorous quality control by an external independent testing institute (routine laboratory) in order to guarantee the respective growth behaviour and the morphology of the metaphases and chromosomes.

General

Prenatal diagnostic requires and is dependent on various invasive and non-invasive techniques in order to examine the fetal nesting and to determine possible fetal genetic abnormalities or malfunctions. The amniocentesis and chorionic biopsy sampling (CVS) represent the essential methods for the invasive diagnostic within the scope of the clinical examination of chromosome abnormalities of a fetus in order to clarify the risk of genetic diseases.

Since amniocentesis became established in the cytogenetic routine and cell cultures supplied the source material for the chromosome analysis and other prenatal genetic examinations, diverse techniques including suitable cell culture media and stimulants have been developed and established, mostly – that means up to now - (almost) always with the use of Fetal Bovine Serum (FBS).

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Product Description

AMNIOPAN has been developed especially for the prenatal In-Vitro Diagnostic of human fetal cells from amniotic fluid and chorion villi biopsy sample material and shall guarantee a standardized application.

The recipe has been optimized by means of rigid usability testing of primary human fetal cells from amniotic fluid and CVS. Here they paid special attention to a quick attachment of the cells to the base of the cell culture bottles as well as to growth optimization in order to enable the diagnosis to be carried out as soon as possible.

Sources of disturbance like induced aberrations and other changes of the chromosome image should be avoided if at all possible res. the tendency, which exists with every stimulated culture of such cells, should be reduced as far as this is technically possible.

Warning Information

An element of risk regarding wrong karyotypes remains in the nature of it, it is always involved and cannot be excluded completely by any producer of cell culture media. In the end it is one of the most important tasks of the tester to pay attention to it. Therefore AMNIOPAN Medium is allowed to be used only by personnel with experience in the judgment of such phenomena.

AMNIOPAN is supplied ready for use. No further supplementation is necessary, as antibiotic, L-glutamine and Fetal Bovine Serum (FBS), hormones and growth factors are already included and this is why AMNIOPAN Medium is very user-friendly for the daily routine application.

AMNIOPAN is buffered with NaHCO_3 and has phenol red as pH-indicator. The formulation which is ready for use reduces the risk of technical errors and the contamination of the cultures.

Storage and Best-by Date

Deep-frozen: Recommended storage at $-20\text{ }^\circ\text{C}$ in the dark until the best-by date which is indicated on the adhesive label of every bottle.

Refrigerated: Storage of opened or defrosted bottles at $2-8\text{ }^\circ\text{C}$ possible for up to two (2) weeks.

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Instructions for Use

AMNIOPAN is ready for use and can be applied immediately after defrosting in the water bath (preferably) at 37 °C or overnight in the refrigerator (alternatively), turning it slightly in order to get a homogenous mixture.

AMNIOPAN shall be stored in the dark at 2-8 °C after opening and shall be used within 2 weeks. Avoid repeated warming up and cooling as well as a long exposure to light.

AMNIOPAN is a complete medium, supplied in deep-frozen condition and filled sterile. An additional filtration is not necessary and it must even be advised against it.

Defrosting

AMNIOPAN should be defrosted preferably at 37°C in the water bath. Afterwards or during the defrosting the bottle has to be turned slightly in order to reach a homogenous distribution of the content before use. When it is defrosted in the incubator with CO₂ – fumigation, a careful opening of the screw caps is recommended in order to reach a pH-balance. Thus the culture start is optimized.

pH- Control

AMNIOPAN is adjusted to a correct pH-value of 6,9 – 7,1 when it is supplied, which can fluctuate up to a point, however.

AMNIOPAN contains phenol red as pH-indicator:

- Dark red indicates a high pH-value (basic). Phenol red indicator turns into dark red at pH 7,4. In this case the medium is too basic and its acid value can be corrected by equilibration. For this purpose the screw cap has to be opened by approx. a quarter and the bottle has to be kept in an atmosphere of 5% CO₂ in the incubator for approx. 1 hour. Here the medium remains sterile under respective sterile conditions and adapts itself automatically to the correct pH-value.
- Yellow indicates a pH-value which is too low. Phenol red indicator turns into yellow at pH 6,7. Yellow medium is too acid and has to be adjusted in the same way as the medium which is too basic.
- Alternatively the culture bottle can be fumigated individually. In this case a sterile 5 % CO₂– fumigation under sterile conditions is necessary.
- The colour can be optically compared with a fresh, newly defrosted bottle in order to determine the correct colour value.

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Antibiotic

AMNIOPAN contains 50 µg/ml gentamycin which is fully identical in its antibiotic effect compared with penicillin – streptomycin, however less toxic for the cells.

Stability

- **AMNIOPAN** remains stable until the best-by date indicated if it is stored correctly (-20°C).
- Do not use after expiry of the best-by date.
- **AMNIOPAN** can be used up to 2 weeks after defrosting if it is stored at 4°C in the refrigerator. L-glutamin may reduce, however. By another sterile addition of L-glutamin up to 2,0 mM (1/100 vol. of 200 mM glutamin stock solution) this can be corrected.
- Repeated warming up / cooling or action of light has to be avoided by all means.

Quality Assurance

AMNIOPAN is tested for sterility, pH-value and osmolality. Additionally to these standard specifications every batch produced is tested for cell growth (clones), attachment (attachment speed), cell number (mitosis rate) and morphology of the metaphases and chromosomes. A quick growing and a high cloning rate of the cells (7 – 10 days) are decisive.

Safety

If anything is unclear please contact the customer service of PAN-Biotech GmbH or an authorized representative.

Exclusion from Use:

- **Packing damaged.**
- **Product cloudy or precipitates visible.**
- **Color not orange – red.**

If the product arrives in defrosted condition, deep-freeze it immediately at -20°C and inform the customer service of PAN-Biotech GmbH or an authorized representative.

FOR USE IN THE IN-VITRO-DIAGNOSTIC BY MEANS OF CULTURE AND GROWTH OF HUMAN FETAL CELLS FROM AMNIOTIC FLUID OR CHORION VILLI BIOPSY SAMPLE MATERIAL (CVS).

ADDITIVES TO THE **AMNIOPAN** ARE NEITHER NECESSARY NOR RECOMMENDED.

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Restriction

Every laboratory has to establish their own test procedures and regularly carry out the tests for the selection and quality assurance of new cell culture media and batches before their use, especially if they are engaged in clinical routine diagnostic.

PAN-Biotech's contribution restricts itself to only the supply of culture medium and therefore a successful application under the special laboratory conditions cannot be guaranteed.

Every produced batch of **AMNIOPAN** is carefully tested by means of primary amniotic cells with the object of ensuring product properties which could be of importance for the application in the In-Vitro-Diagnostic.

Besides, PAN-Biotech does not take any guarantee and especially not for the diagnostic, which is based on many individual components and only indirectly susceptible to the properties of the culture medium.

Because of the complex situation PAN-Biotech cannot take any liability for damages too which can go back to the cell growth.

Because of necessary precautionary measures:

- EVERY CLINICIAN AND SCIENTIST MUST FORM HIS OWN INDEPENDENT OPINION ON THE SUFFICIENT PROPERTY FOR THE USE IN THE IN-VITRO-DIAGNOSTIC IN HIS LABORATORY.
- PAN-BIOTECH GMBH DOES NOT TAKE ANY GUARANTEE ON A SUCCESSFUL DIAGNOSIS ONLY BASED ON THE APPLICATION OF PAN-BIOTECH MEDIUM.
- PAN-BIOTECH'S CONTRIBUTION IS ONLY IN THE SUPPLY OF CELL CULTURE MEDIUM WHICH HAS BEEN PRETESTED FOR THE INTENDED PURPOSE AND WHICH SHOULD THEREFORE BE APPROPRIATE.

PAN Biotech's cell culture products are produced under standardized manufacturing conditions which are established and usually applied for such a kind of production.

The risk of a contamination or fluctuation of properties corresponds to the statistic risk of methods of the sterile production res. production on the basis of raw materials of animal or human origin.

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Cell Culture Methods

The records stated here shall offer a clue for AFC and CVS cultures by using **AmnioPAN Medium**.

The methods can be replaced by others, modified or partly exchanged, depending on the availability of optimized or other procedures.

Most cytogenetic laboratories have their own methods and use the cell culture medium within the scope of their own methods and conditions.

Thus **AmnioPAN Medium** is a substitute in applied procedures. Most common cell culture methods are based on a so-called „OPEN“ system.

OPEN SYSTEM / SEALED SYSTEM

Definition „open“ system: Cultures grow in plates with ventilated covers or in bottles / tubes with loosened or ventilated caps in 5% CO₂ atmosphere (incubator with fumigation) in order to guarantee a respective gas exchange.

Definiton „sealed“ system: Cultures grow in a hermetically sealed system in a standard incubator without fumigation by air – CO₂ mixture. Here it is useful to think about a suitable buffering of the culture medium.

RECOMMENDATIONS FOR THE USE OF „OPEN“ SYSTEMS

In Situ Method

1. Concentrate the cell by centrifugation of the amniotic fluid at a low number of revolutions.
2. Remove approx. 90-95% of the supernatant and resuspend the cell pellet in the remaining volume of the patient's own amniotic fluid. Dilute the cell concentrate with a volume of AmnioGrow Plus Medium in order to get a plating volume of 0,5 ml per coverslip (total 4 coverslips) or 2 ml per flask.
3. Incubate the culture medium at 37°C in 5% CO₂ atmosphere.
4. Add 2 ml AmnioGrow Plus Medium to every culture on day 2.
5. After 4 – 5 days test the cultures for growth. Feed the cultures after growth has been determined. In order to feed the cultures, carefully suck off the used medium and replace it by 2 ml fresh AmnioGrow Plus Medium. It is recommended to repeat this procedure every two days.
6. Test the culture by analogy after day 5 and „harvest“ as soon as sufficient colonies can be observed.
7. In order to achieve the best results, feed the cultures with AmnioPAN Medium on the day before the harvest.

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Bottle Method

1. Concentrate the cells by centrifugation of the amniotic fluid at a low number of revolutions.
2. Remove 95% of the supernatant and resuspend the pellet in the remaining fluid (patient's own amniotic fluid). Resuspend the concentrate with sufficient AmnioPAN Medium in order to get 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% CO₂ atmosphere.
4. On day 5 test for growth. Remove used medium and replace it by fresh one. Harvest as soon as there is sufficient cell growth.
5. Test the cultures for growth and then change the medium daily until sufficient colonies have developed so that you can harvest.
6. For best results feed the cultures with AmnioPAN Medium especially on the day before the harvest. This sets off a wave of division and produces plenty of mitoses.

RECOMMENDATIONS FOR THE USE OF „SEALED“ SYSTEMS

AmnioPAN Medium can be used in „sealed“ systems as long as the pH-value is physiologically maintained (pH = 6,9 to 7,4). Sealed systems are dependent on an adequate buffer capacity of the medium.

Sealed systems work preferably in cloning procedures with low cell number as higher cell densities produce a larger number of acid metabolites which can “acidify” the medium so much that it gets into an unphysiologic range.

Some information on „sealed systems“:

- Method 1: Add 2% (v/v) sterile 1,0 M HEPES stock solution to **AMNIOPAN**. The sterile 1,0 M HEPES solution must be adjusted to pH 7,0 at 20 °C with 1,0 M NaOH. Then the cells are added to the HEPES-supplemented medium and incubated in a sealed culture dish at 37 °C.
- Method 2: Pre-equilibrate the culture bottle with **AMNIOPAN** and the cells in a 5% CO₂ incubator for one hour before sealing and the culture at 37 °C.
- Method 3: Fill every individual bottle with **AMNIOPAN** and cells with 5% CO₂-95% air mixture by means of a sterile pipette (sterile filter interposed) for approx. 20 sec. Then seal the system hermetically and cultivate it at 37 °C.

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Growth Problems

- poor growth is dependent on several factors

- Cell number too low (cells per milliliter); this is among other things dependent on the pregnancy week
- Quantity of amniotic fluid, the less the fluid the slower res. the worse the growth
- Conditions at the sampling (sterile conditions)
- Conditions at the transport (the faster the mixture can be prepared the better). 7 days after the preparation of the culture at least one to two colonies should be there.
- „Dirty“ cultures (debris) grow slower as a rule. The cell debris can be far reduced by exchanging the medium completely after the first growth controls and by washing the culture with PBS-buffer.
- If the amniotic sample is already visibly bloody, the quantity of erythrocytes can considerably disturb the growth. The following procedure has proved successful: complete change of the medium when “feeding” the first time and complete change of the medium including the washing with PBS when “feeding” the second time.

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References

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Further Information

For further information about this and / or other PAN-Biotech products please contact the info service tel.: +49 8543 6016-30

Out of Germany please refer to the PAN-Biotech product information for the local hotline in your area.

Contact the responsible sales representative. Please take the contact data from the PAN-Biotech homepage or ask us.

For In-Vitro-Diagnostic Use.

Attention: Not suitable for human or animal therapeutical use. Use for other purposes or unlike prescribed is inadmissible.

EVERY USER MUST ORIENT HIMSELF BY THE CURRENT PRODUCT INFORMATION WHICH IS ALWAYS CALLABLE BY THE MANUFACTURER.

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