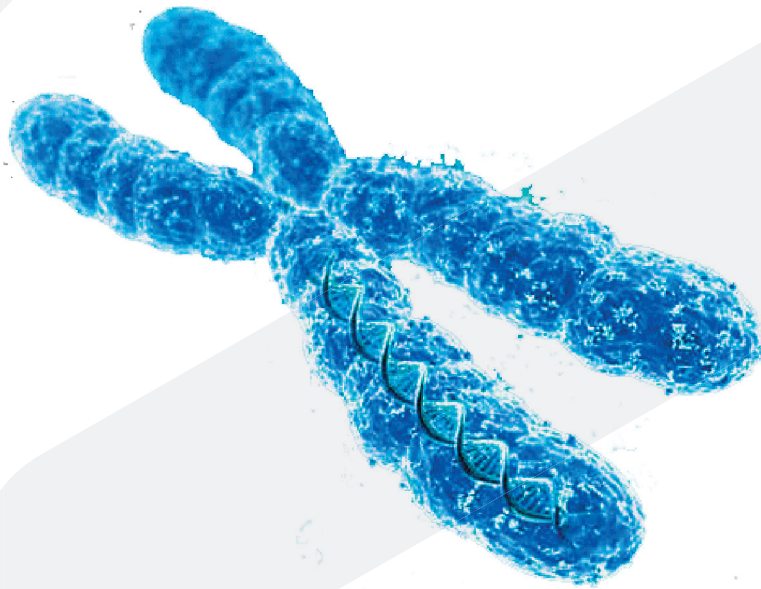


NEW PRODUCT !!!



Amniomed® PLUS

**An enhanced complete medium for
Amniotic Fluid Cells and Chorionic Villus Samples**

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EuroClone®

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Product Information

Amniomed® Plus is used in the establishment of primary in vitro cultures of foetal human cells from amniotic fluid, which may be used in karyotyping, Fluorescent In Situ Hybridization (FISH) and other cytogenetics procedures. This new improved formulation has been optimized by providing end-user with enhanced plating and efficiency in foetal cell culture. In addition, this product supports more efficient and fast cell attachment and growth, resulting in earlier chromosome analysis. **Amniomed® Plus** has also proven to be an excellent medium for foetal tissue culture from Chorionic Villus Sampling (CVS).

Formulation

Amniomed® Plus is a complete medium (1X), ready to use, with foetal bovine serum (FBS), L-Glutamine, antibiotics, growth factors and a more efficient buffering system.

Amniomed® Plus is developed for either *in situ* and flask system.

Storage

Frozen: Store at -5°C to -30°C up to 18 months.

Refrigerated: Store at +4°C for one week (7 days).

Protect from light.

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

Usage and reconstitution

Amniomed® Plus should be thawed at refrigerator temperatures (2 to 8°C) or in a 37°C water bath with frequent swirling. In either case the media should be mixed gently after thawing.

Substitute **Amniomed® Plus** for the media you are presently using. No further additions are required.

Activity

Amniomed® Plus is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each batch is tested for cell growth.

Format

Amniomed® Plus is a complete medium, provided in a liquid form, frozen.

Stability

Amniomed® Plus remains stable up to 18 months when frozen and can be re-frozen as aliquots (keeping the pH stable is the main problem because the tubes may remain uncapped too long in the hood). Once thawed **Amniomed® Plus** can be stored at + 4°C for one week.

Amniocyte Culture "In situ" Protocol

This protocol provides a guide for human amniocyte culture using **Amniomed® Plus**. It can be used to replace either part or the whole of existing protocols for amniocyte culture at the user's discretion.

"In situ" Cell Culture

- a) Transfer the amniotic fluid sample (about 20 ml) in 2 sterile centrifuge tubes and spin at 1200 rpm for 10 minutes
- b) Decant the supernatant for Alpha-fetoprotein analysis, leaving about 1 ml in each tube.

*Please Note: Thaw the **Amniomed® Plus** in a 37°C waterbath and mix well by swirling prior to use. Addition of L-Glutamine, antibiotics and serum are not necessary since these components are already present.*

- c) Prepare 4- 6 sterile Petri dishes (35 mm diameter) containing round glass slide (AMNIODISH cod. EK AMN-240)
- d) Distribute cell suspension into Petri dishes.
- e) Add 2 ml of **Amniomed® Plus** to each Petri dish.
- f) Put the cultures in a 37°C at 5% CO₂ incubator.
- g) On the 5th day observe carefully the cell growing condition and then replace the medium with 2 ml of fresh one.
- h) One or two days later observe the cultures and if the cell colony growth is adequate add Colcemid solution at the final concentration of 0.05µg/ml.

Maintain in incubator for 2-4 additional hours before chromosome preparation.

Flask method culture

(Use the same procedure as for the above "in situ" culture with the following adaptation)

- a) Distribute cell suspension into 2-4 flasks
- b) Add to each flask 4-5 ml of **Amniomed® Plus**
- c) Place cap loosely on the flask and put the cultures in a 37°C at 5% CO₂ incubator

Chromosome preparation

- a) Decant the medium completely by tilting the plate and suctioning from the corner
- b) Add 3.0 ml of hypotonic solution in each Petri dish and maintain at room temperature for 10 minutes
- c) Add 0.5 ml of fixative mixture (3:1 methanol – acetic acid) directly to the hypotonic solution and leave for 5 minutes
- d) Decant off the supernatant and immediately add 3.0 ml of fresh fixative
- e) Repeat twice to remove carefully any trace of residual water present in the Petri dishes (The duration of these washings has no influence on final result).
- f) Remove coverslip from the dish and immediately put it to dry in OPTICHROME device (Optichrome cod. EK AMH-950) at right condition of temperature and relative humidity.

Chorionic Villus Sampling (CVS) Culture Protocol

- a) Transfer the specimen from the transport tube to a 60 mm Petri dish containing 5 ml of RPMI 1640
- b) Wash Villi with fresh medium to remove the blood cells
- c) Using the inverted microscope, carefully dissect and remove any remaining clots or decidua from Chorionic Villi.
- d) Wash carefully Villi in Hank's Balanced Salt Solution without Ca^{++} and Mg^{++} .

Long-term cultures

- a) Transfer the Villi in a sterile centrifuge tube containing 2 ml of Pronase E (Merk, 4.000.000 PU/g) * and incubate for 4-6 minutes at room temperature, gently shaking from time to time
- b) Add 3 - 5 ml of cold HBSS (without Ca^{++} and Mg^{++}) to stop enzyme action
- c) Centrifuge for 5 minutes at 1500 rpm and discard the supernatant
- d) Add 2 ml of sterile Collagenase type II (Sigma, 1 mg/ml) and incubate at 37 °C for 10 minutes
- e) Add 3 - 5 ml of cold HBSS to stop the enzyme action
- f) Centrifuge for 5 minutes at 1500 rpm and discard the supernatant
- g) Add 2 ml of **Amniomed® Plus** and resuspend the pellet
- h) Prepare 2 to 6 Petri dishes (Amnidish) depending on the volume of cell pellet. Add 2 ml of **Amniomed® Plus** medium to each Petri dish and distribute the cell suspension
- i) Incubate at 37 °C in a 5% CO_2 incubator
- l) After 4 days of culture, check for progress of growth using an inverted microscope
- m) When the areas of growth show a number of cells in mitosis, add one drop of Colcemid to each Petri dish (at a final concentration of 0.05 $\mu\text{g}/\text{ml}$) and keep in incubator for additional 4 – 6 hours.

Chromosome preparation

Follow the same procedure described in "Chromosome preparation" for Amniotic Fluid cell culture.

* Dissolve 25 mg of Pronase E in 25 ml of HBSS without Ca^{++} and Mg^{++} . Filter with a 0.2 μm membrane. Aliquot and put in deep freezer (- 20°C).