PRODUCT SPECIFICATION

Amniomed Plus

Cat. No. EK AMG-200

Product description

Amniomed Plus is used in the establishment of primary cultures of human amniotic fluid cells, which may be used in karyotyping, Fluorescence *In-Situ* Hybridisation (FISH) and other cytogenetic procedures. This formulation has been optimised by providing a sterile, 1 x frozen medium that reduces the chance of technical error and / or culture contamination. In addition, this product supports more efficient cell attachment and cell growth, resulting in earlier chromosome analysis. Amniomed Plus has also proven to be an excellent medium for use in CVS, foetal tissues and skin fibroblast cultures.

Amniomed Plus is a complete medium, provided in a frozen, sterile format. Re-filtering the media is not necessary but will not alter its performance. Of course, successful cell cultures depend on the use of good sterile technique. The following suggestions may be useful in using Amniomed Plus.

Components

Amniomed Plus is a complete medium, ready to use, with foetal bovine serum (FBS), antibiotics and other active ingredients already included.

Basal medium	**DMEM/HAM'S F-12 (1:1)**
Buffers	25 mM Hepes
Serum	Foetal Bovine Serum
Antibiotics	Gentamycin sulphate
Aminoacid	L-Glutamine
pH indicator	Phenol red
Other components	Growth factors and hormones

Storage/Stability

Amniomed Plus should be stored in the dark at freezer temperature (-20°C). In this condition, Amniomed Plus remains stable for two/three years. Amniomed Plus should be thawed in a 37°C water bath and mixed by swirling prior to use. Some laboratories thaw media in a 37°C CO_2 incubator with the lid slightly opened, to allow for automatic pH normalisation. Warm media at the proper pH is best for the initialisation of amniocyte cultures.
After thawing, the medium should be kept at refrigerated temperature (2-8°C). Discard the medium within 8 days after thawing.

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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 optimal protocols for amniocyte culture at the user's discretion. The majority of cytogenetic laboratories have used Amniomed Plus as a complete medium without any alteration to their present protocols.

Culturing cells

a) Transfer the amniotic fluid to sterile tubes and centrifuge the cells at 1000rpm x 10'.
b) Decant the supernatant for Alpha-fetoprotein analysis.
c) Resuspend the cell pellets in 1.5ml Amniomed Plus.

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

d) Place 0.5ml of cell suspension gently onto each sterile Petri dish 35 mm diameter, contains round glass slide (Amniodish cod. EK AMN-240)
e) Incubate the plates in a 37°C incubator with minimal mechanical disturbance for 48 hours. After 48 hours (Day 2), add 2.0ml of fresh Amniomed Plus.
f) After 4 days (Day 4), replace the culture medium with 2.0ml of fresh Amniomed Plus.
g) On the 5^{th} / 6^{th} day or when growth is adequate, add 1 drops of Colcemid solution (10mg/ml) to each 2.0ml of culture medium.
h) Incubate the culture for 60 - 90 minutes at 37°C.

Chromosome preparation

a) Decant the medium completely by tilting the plate and suctioning from the corner. Be careful not to disturb the coverslip.
b) Add 3.0ml of a hypotonic solution (sodium citrate 1%) in each Petri dish, and leave the plate undisturbed at room temperature for 10 minutes.
c) Add 0.5 ml of fixative (3:1 methanol : acetic acid) directly to the hypotonic solution and leave undisturbed at room temperature for 5 minutes.
d) Suction off the supernatant, then add 3.0ml of fresh fixative at room temperature (the time for this wash has no influence on final results).
e) Repeat f) once.
f) Remove coverslips from the dishes and dry under constant condition of right temperature and relative humidity into Optichrome (cod. EK AMH-950).
g) Once the coverslips are dry, mount them on a standard glass slide with mounting medium and let dry at room temperature for about 15-20 minutes. Once dry, heat them at 60°C for 2 hours.
h) Chromosomes are now ready to be stained for banding and work-up.

Chorionic villi sampling (CVS) culture protocol

a) Transer the specimen from the transport tube into a 60 mm Petri dish containing 5 ml of RPMI 1640
b) Wash villi with fresh medium to remove blood cells
c) Using the inverted microscope, carefully dissect any remaining clots or decidua fragments

Long-term culture

a) Transfer the villi in a 15 ml sterile centrifuge tube containing 1 ml of Pronase E (Meerk, 4.000.000 PU/g) and incubate at room temperature for 4 - 6 minutes. This step should detach the outer layer (syncytiotrophoblast) cells of the chorionic villi
b) Add 3 - 5 ml of cold Hank's Balanced Salts Solution (HBSS must be kept at +4°C)
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

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e) Add 3 - 5 ml of cold Hank's Balanced Salts Solution (HBSS must be kept at +4°C)
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 Amniodish depending on the number of cells. Add 2 ml of Amniomed Plus medium to each Petri dish. Place about 500 µl of the cell suspension on each Petri dish.
i) Incubate at 37°C in 5% CO_2
l) After 4 - 5 days of culture, check for progress of growth using an inverted microscope
m) When the areas of growth show a number of cells in mytosis equal three to four microscope fields (100X), add one drop of Colcemid to each Petri dish and keep at 37 °C in 5% CO_2 for additional 4 - 6 hours

Chromosome preparation

See protocol chromosome preparation of Amniotic fluid.

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