CAUTION

Handle in accordance with established bio-safety practices.

Designated Purpose
Amniogrow Plus serves the cultivation and growth of human amniotic cells and chorionic villi and is to be used exclusively for *in vitro* diagnostic purposes on samples taken from humans.

Composition
Basal media, pretested FBS, hormones and growth factors. Buffered with HCO^3.
Contains Gentamycin (50 µg/ml) and L-Glutamine.

Shelf Life and Storage
Amniogrow Plus is stable for 2 years after production when stored at ≤-18 °C. Due to quality and sterility Amniogrow Plus should be used within a maximum of 2 days after opening and storing at +2 °C to +8 °C.

Thawing
Amniogrow Plus medium can be thawed at +2 °C to +8 °C overnight or in a +37 °C water bath. Mix medium thoroughly by swirling prior to use. In case of pH variation (pink or yellow) open cap slightly (about ¼ turn) and incubate bottle in a 5% CO^2 incubator until normal coloration (phenol red). Amniogrow Plus does not contain components sensitive to pH changes of +/- 2. Warm medium at the proper pH is best for the initialization of cultures.

Protocol
The protocol below provides guidance for amnion and chorionic villi cell culture using Amniogrow Plus. The medium is bottled under aseptic conditions. The maintenance of sterility is absolutely necessary for the use in *in vitro* diagnostics and must be strictly adhered to by the user. This high quality medium can naturally be used within established procedures. It is up to the user to adopt either parts or all of the optimized protocol described below.

In situ Method
- Concentrate the cells by centrifugation of the amniotic fluid at low speed.
- 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 Amniogrow Plus medium to a volume of 2 ml to allow a final plating volume of 0.5 ml per coverslip (total of 4 coverslips).
- Incubate cultures at +37 °C in a 5% CO^2 atmosphere.
- Add 2 ml of Amniogrow Plus medium to each culture on day 2.
- 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 Amniogrow Plus medium. Recommendation: feed cultures every 2 days.
- Check cultures for growth after 5 days, and harvest when sufficient colonies are observed.
- For best results, feed cultures with Amniogrow Plus medium the day before the harvest.

Flask Method
- Concentrate the cells by centrifugation of the amniotic fluid at low speed.
- 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 to a minimum of 2 ml with prewarmed Amniogrow Plus medium to allow a final plating volume of 2 ml per flask.
- Incubate cultures at +37 °C in a 5% CO^2 atmosphere.
- On day 5 check for growth. Remove medium and replace with fresh Amniogrow Plus medium and harvest if sufficient cell growth is observed.
- Completely exchange exhausted medium every other day until harvest.
- For best results, feed cultures with Amniogrow Plus medium the day before the harvest.

Important Remarks
- For *in vitro* diagnostic use only (IVD)
- CAUTION: Not for human or animal therapeutic use. Uses other than the intended use may be a violation of local law.
- Each laboratory must carry out their own testing procedures on new media according to national legislation prior to releasing them to the lab for routine *in vitro* applications.
- Each clinician/scientist must make an independent judgment on whether this medium is suitable for use in *in vitro* diagnostic applications conducted in their laboratory.
- Cytogen GmbH does not guarantee the successful outcome of any diagnostic testing based solely on the use of Cytogen brand medium.

CE marked
With Amniogrow Plus, Cytogen offers a CE marked medium for IVD which fulfils the requirements of the directive 98/79/EC defined by the European Commission.

MANUFACTURER

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