

Attention: The patient samples are biological material and therefore safety precautions are necessary.

Purpose of Use

Amniogrow Plus medium 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

It is a complete medium composed of basal-medium, pre-tested FBS, hormones and growth factors for the corresponding cell or tissue type, phenol red, NaHCO₃, plus gentamicin and L-glutamine.

Shelf Life and Storage

Amniogrow Plus medium is durable at a storage ≤-18 °C for 18 months from the date of manufacture. For quality and sterility reasons, the use of Amniogrow Plus medium is recommended after opening at a storage of + 2 °C to + 8 °C of a maximum of 7 days. Repeated thawing and freezing should be avoided. Amniogrow Plus medium not exceed the expiry date indicated on the label.

Thawing

Thaw Amniogrow Plus medium at + 2 °C to + 8 °C overnight. Thawing in a water bath at 37 °C is not recommended. Mix well before using Amniogrow Plus medium. The normal pH value is 7,2 as indicated by the phenol red indicator. In the case of a pH deviation (yellow or pink), the pH value is obtained by incubating the slightly open bottle (approx. ¼ rotation of the lid) in a 5% CO₂ incubator equilibrated until the medium has reached the normal color red. Amniogrow Plus medium contains no components whose quality is affected by pH fluctuations of +/-2. Heated medium at 37 °C and correct pH-value ensures an optimal start of the culture.

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.

Bottle Method

- Concentration of cells by centrifugation at low speed
- Take off 90 – 95% of the Supernatant and resuspend cells in the remaining residue.
- Dilute the pellet with preheated Amniogrow Plus medium to at least 2 ml to obtain 2 ml per culture bottle
- Incubate at + 37 °C and 5% CO₂ in incubator
- Check growth on the 5th day and replace the medium with fresh Amniogrow Plus medium
- Replace the used medium to the harvest on a regular basis
- For optimal results medium the day before harvest change

in situ method

- Concentration of cells by centrifugation at low speed
- Remove 95% of the Supernatant and resuspend cells in the remaining excess
- Dilute the cell suspension with preheated Amniogrow Plus medium to at least 2 ml to obtain 0.5 ml suspension per deck of glass (total number 4)
- Incubate at + 37 °C and 5% CO₂ in incubator

- 2 ml Amniogrow Plus medium on the second day
- Control cell growth after 4 – 5 days
- Immediately afterwards, gently vacuum the entire medium and replace with 2 ml preheated fresh Amniogrow Plus medium
- Recommendation: Media change every 2 days
- For optimal results replace the medium the day before harvest

Important observation

- Calciumoxalat crystals may occasionally form, but have not yet shown any negative effects on cell growth.
- Thawing in a water bath at 37 °C should be avoided since precipitates can form.

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 medium, Cytogen offers a CE marked medium for IVD which meets the requirements of Directive 98/79 / EC, established by the European Commission

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