

Sample Preparation

Chorionic villus Biopsy and Arbot Material Samples must be freed from maternal material. In Corionic villus is the Dizidua available. Then everything is crushed with a sterile scalpel, and now you can cross out this Material in a Culture Flask (Vial). In a second Method, one uses the Enzyme Dispase to digest for 20 Minutes at an adequate Concentration. The Cells are kept for a time in a humid Chamber, the Culture Bottle is turned upside down and then turned slowly. **Cautious** So that while the material does not come off!

Use of biological Material with proper safety Precautions: Warning Caution.

Introduction

Chorionic Villus vultures are routinely used for cytogenetic analysis of cells derived from chorionic villus sampling (CVS) and spontaneous abortions. Since chorionic villi cells are derived from the fertilized egg, they have the same genes as the fetus. Culturing these cells requires specialized media for optimum results and PrenaPlus culture medium was designed specifically for this purpose.

Media Composition

PrenaPlus is a complete sterile filtered medium containing basal nutrients pre-tested FBS, hormones and growth factors for CS cells, phenol red (for pH indication) NaHCO₃, gentamicin and sustained effects L-glutamine. No additives are required for efficient cell attachment and growth..

Storage and Stability

PrenaPlus Medium is stable for more than 2 years when stored frozen (-18°C or colder). For optimal performance and sterility, it is recommended that PrenaPlus be used within 2 days after thawing and storage of 2°C to 8°C.

Thawing

PrenaPlus Medium can be thawed quickly in a 37°C water bath or in a refrigerator between 2°C to 8° overnight. Thawing in a microwave oven is not recommended. Mix the medium will by gently swirling. The normal pH is 7.2 as indicated by the intrinsic red phenol red indicator. However, if pH variation is observed (yellow=too low, violet=too high) the pH can be normalized by incubating the slightly opened bottle (about 1/4 turn of the cap) in a 5% CO₂ incubator. None of the PrenaPlus Medium components are affected by pH fluctuations of +-2 pH units. Initiation of cultures with medium at 37°C and pH 7.2 will insure optimal start of the culture.

General Culture Protocol

PrenaPlus Complete Media can be used for In situ and Direct Culture methods. The methods described below are a guide for the use of PrenaPlus Medium in the cultivation of appropriate cell samples. PrenaPlus Medium is filled under aseptic conditions. The preservation of the sterility of the product is necessary for use in In Vitro diagnostics and must be strictly adhered to. PrenaPlus is a robust, high performance medium that is highly adaptable to current protocols being used in specific laboratories. A more detailed suggested protocol is available on request from Cytogen.

Abbreviated *in situ* method

- Concentrate cells by centrifugation at low speed
- Remove 95% of supernatant and resuspend cells in the remaining amniotic fluid – 90
- Dilute the cell suspension with preheated Prenaplus medium at least 2 ml, to obtain 0.5 ml oral suspension per coverslip (Total 4)
- Incubate at +37 ° C, 5% CO₂ humid incubator
- On the second day add 2 ml Prenaplus
- Check at 5 days for growth of cells
- Immediately thereafter gently aspirate the entire medium and replace with 2 ml pre-warmed fresh medium Prenaplus
- Recommendation: media change every 2 days
- Change for optimal results media on the day before the harvest

Abbreviated Direct Method

- Concentrate cells by centrifugation at low speed
- Remove 95% of supernatant and resuspend cells in the remaining amniotic fluid. Do not vortex.
- Cell suspension with preheated PrenaPlus of least 2 ml diluted to obtain 2 ml per culture bottle
- Incubate at +37 ° C, 5% CO₂ incubator
- On the 5th day check growth and replace the medium by fresh PrenaPlus Medium.
- Replace Spent medium to harvest regularly
- Change For optimal results media on the day before the harvest.

Important Notes

- Always handle biological materials with proper safety precautions.
- Exclusively for *In vitro* Diagnostics (IVD).
- Warning: Not suitable for therapeutic use in humans or animals. PrenaPlus Media is for diagnostic purposes only.
- Each laboratory is required to carry out representative tests according to regulations before the media can be used in routine diagnostics.
- Acceptance of PrenaPlus as a diagnostic reagent is at the sole discretion and responsibility of the testing laboratory and responsible management.
- Cytogen GmbH does not guarantee success of diagnostic testing solely through the use of Cytogen products.

CE marking

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

MANUFACTURER

Cytogen - Products for Medical Research GmbH,
Langgasse 73, D-35576 Wetzlar, Germany
Tel. +49 6441 679 55 88
fax +49 6441 679 55 89
Email: cytogen@eurobiz.de
Web: www.cytogen.info

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