


KREAvital Prenatal Medium PLUS (Complete)

Complete Medium for Human Amniotic Fluid and Chorionic Villi Samples

Cat. No: KBI-90013 (100 ml)

Store at:  -20°C

Product Description

The in vitro cultivation of amniotic fluid cells and chorionic villi is an essential part of every diagnostic cytogenetics laboratory, since the preparation of metaphase chromosome spreads is dependent upon obtaining cells in division. KREAvital Prenatal Medium PLUS is specifically optimized for the primary culture of human amniotic fluid cells and chorionic villi samples used in prenatal diagnostic testing. The medium is a specialized version of KREAvital Prenatal Medium (Complete), as it has enhanced buffering capacity both in open (CO₂) and closed systems; it has increased metaphase yield of a highly pure amniotic cell population, yielding less epithelial cells; it has extended stability at 2 - 8°C. The medium is supplied frozen and contains serum, glutamine and antibiotics.

Precaution and Disclaimer

1. For in vitro diagnostic use. The medium is not intended for therapeutic use.
2. Do not use if a visible precipitate is observed in the medium.
3. Use of KREAvital Prenatal Medium PLUS does not guarantee the successful outcome of any chromosome analysis testing.
4. Do not use KREAvital Prenatal Medium PLUS beyond the expiration date indicated on the product label.
5. Due to the residual risks of sample contamination, and the severity in such case, we recommend to establish parallel test systems as part of the procedure.
6. In case of questions please contact your local sales representative or send an email to customerservice.amsterdam@leicabiosystems.com

Storage and Stability

KREAvital Prenatal Medium PLUS should be kept frozen at -10 to -20°C.

After thawing, the medium should be stored at 2 - 8°C. The medium should be used within 14 days after thawing. In case smaller quantities are needed, use aseptic techniques to dispense into aliquots so repeated freezing and thawing are avoided.

Protect the medium from light.

Instructions for Use

Thaw KREAvital Prenatal Medium PLUS Medium at refrigerator temperatures (2 - 8°C). Mix gently after thawing.

Note that the medium already contains L-Glutamine and antibiotics.

Procedure

KREAvital Prenatal Medium may be used for:

- Primary culture of amniotic fluid cells
- Culture of passaged amniotic fluid cells
- Propagation of chorionic villus cells

The medium may be used in both open and closed culture systems.

The following protocol and the volumes indicated are only general guidelines for use.

In Situ Culture of Amniotic Fluid Cells

1. Centrifuge 20 ml of amniotic fluid at 750 rpm for 10 minutes.
2. Carefully decant the amniotic fluid from the cell pellet into a sterile test tube.
3. Re-suspend the cell pellet with 2 ml of amniotic fluid.
4. Add 2 ml of KREAvital Prenatal Medium and swirl gently.
5. Culture 0.5 ml of the cell suspension on each coverslip in a tissue culture dish.
6. Incubate cultures at 37°C in 5% CO₂ atmosphere.
7. Flood cultures on day 2 with 1.5 ml of KREAvital Prenatal Medium.
8. After 5 days, check the cultures for the presence of colonies.
9. After the colonies first appear (5-7 days), replace the medium with fresh KREAvital Prenatal Medium.
10. When the cultures have colonies of sufficient size, proceed with harvesting. It is recommended to use cells from 2.5 ml of amniotic fluid per one coverslip.

Note: It is recommended to replace the medium with fresh KREAvital Prenatal Medium the day before harvesting.

Flask Method Culture of Amniotic Fluid Cells – Open and Closed Systems

Use the same procedure as for the in-situ culture, with the following adaptations:

1. Re-suspend the cell pellet with 4 ml of amniotic fluid. Add 16 ml of KREAvital Prenatal Medium and swirl gently.
2. Culture 5 ml per each T25 flask. Place the cap loosely on the flask and incubate undisturbed at 37°C in 5% CO₂ atmosphere.
3. For closed systems: Flush each culture flask with 5% CO₂ – 95% air through 0.2 µm sterile filter for 20 seconds. Tighten the caps and incubate the flasks at 37°C.
4. Check all flasks for growth after 5 days.

Quality Control

KREAvital Prenatal Medium is tested for sterility, pH and osmolality. In addition, each batch is tested for cell growth using primary human amniotic fluid cells.

