

# HiAmnioXL™ Amniocyte Karyotyping Medium

With L-Glutamine, FBS, Gentamicin Sulfate and Sodium bicarbonate  
1X Liquid Karyotyping Medium

**Product Code: AL202A**

## Intended Use:

HiAmnioXL™ Amniocyte Karyotyping Medium is a karyotyping medium recommended for culture and genetic analysis of human amniotic fluid, cells and chorionic villi (CV) samples.

## Principle and Interpretation:

Cytogenetic studies include metaphase and pro-metaphase studies carried out on bone marrow cells to detect chromosomal aberrations associated with structural and numerical abnormalities.

AL202A is HiAmnioXL™ Amniocyte Karyotyping Medium composed of a basal medium and supplemented with L- Glutamine, FBS, Gentamicin Sulfate and Sodium bicarbonate. It is a complete medium and does not require supplementation with any additional component.

## Type of Specimen:

Clinical samples – human amniotic fluid, cells and chorionic villi (CV) samples

## Warning and Precautions:

*In Vitro* Diagnostic Use only. Read the label before opening the container. Wear protective gloves /protective clothing /eye protection /face protection. Follow proper aseptic techniques while handling specimens and cultures. Standard precautions as per established guidelines should be followed while handling clinical specimens. Safety guidelines may be referred in individual safety datasheets.

## Directions:

### A. Flask Method-

1. Add 5ml per each T25 flask. Place the cap loosely on the flask and incubate undisturbed at 37°C in 5% CO<sub>2</sub> atmosphere for 15-20 days till sufficient colonies are observed.

2. When sufficient cells are observed under inverted microscope (once cells reach 70 - 80% confluence), harvest the cells using pre-warmed Trypsin-EDTA solution and proceed with karyotyping.

### B. *In situ* Method-

1. Centrifuge 20mL of amniotic fluid at 750 rpm for 10 mins and transfer the supernatant amniotic fluid to a sterile test tube.
2. Resuspend the cell pellet in 2ml of amniotic fluid and gently mix the suspension by swirling.
3. Place a coverslip in a tissue culture dish and carefully culture 0.2mL of amniotic cell suspension on a coverslip.
4. Incubate the tissue culture dish undisturbed at 37°C in 5% CO<sub>2</sub> atmosphere.
5. After 48hrs, flood the culture with HiAmnioXL™ Amniocyte Karyotyping Medium.
6. Observe the culture for cell growth and colony formation under inverted. Once the colonies start appearing, replace with fresh HiAmnioXL™ Amniocyte Karyotyping Medium media and culture until sufficient colonies observed.
7. After sufficient colonies are observed, proceed with karyotyping.

### C. Karyotyping procedure -

1. Add 50µl of 10µg/ml of Colchicine / Colcemid® (TCL062 / TCL074 / TCL133) and incubate at 37°C for additional 20 minutes.

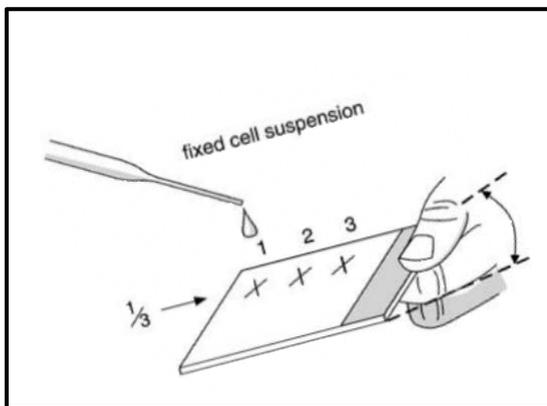
*Note: Incubation of 2 hours gives higher mitotic count than 20 minutes. Users are advised to decide incubation time as per their need and convenience.*

2. After incubation with colchicine, centrifuge the tubes at 1000rpm for 10 minutes.
3. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
4. Add 5ml 0.075M Potassium chloride solution (TCL040) and incubate at room temperature for 20 minutes at 37°C keeping the tubes in an upright position. Mix by inverting.
5. Centrifuge the tubes at 1000rpm for 10 minutes.
6. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
7. Add 1ml of freshly prepared ice cold fixative drop by drop

(Acetic acid: methanol, 1:3 parts) and mix gently by inverting.

*Note: Addition of fixative for the first time may create turbulence which in turn may lead to cell breakage and irreversible clumping. Hence, fixative addition for the first time should be done dropwise and slowly.*

8. Repeat steps 7, 8 and 9 two more times.
9. Resuspend the pellet in 0.5ml of fresh fixative and store them at -20°C till slide preparation.
10. Clean the slides with mild detergent and wash thoroughly under tap water to make them grease free.
11. Place the clean slides in a beaker containing water such that they are completely immersed in water. Keep the beaker in a refrigerator at 2 – 8°C and allow the slides to cool.  
*Note: Steps 12 and 13 can be performed during incubation period of 2-4 hours with colchicine solution to save time.*
12. Mix the cell suspension gently by pipetting up and down. DO NOT vortex.
13. Hold the ice cold wet slide at 45° angle and drop 50µl suspension at the bottom of slide with the help of micropipette in such a way that the suspension hits hard on the slide and then runs down surface. Refer the figure mentioned below.



14. Heat fix the slides by holding them over a hot plate for 10 – 12 seconds, with chromosome spreads facing up.
15. Stain the slides with required staining solution.

### Materials required but not provided:

HiKaryoXL™ Colchicine Solution (TCL062) or  
HiKaryoXL™ Colcemid® Solution (TCL074)  
HiKaryoXL™ Colcemid® Solution (TCL133)  
Potassium Chloride solution 0.075M (TCL040) Methanol  
Acetic Acid  
Giemsa Stain (TCL083)

### Limitations:

Not applicable.

### Quality control:

Appearance  
Orangish colored, clear solution

### pH

7.00 -7.60

### Osmolality in mOsm/Kg H<sub>2</sub>O

340.00 -380.00

### Sterility

No bacterial or fungal growth is observed after 14 days of incubation, as per USP specification.

### Performance Test

Passes.

### Storage and shelf life:

Store at -20°C in a freezer that is not self-defrosting. Once thawed, the product is stable for about 30 days at 2 – 8°C. Repeated freezing and thawing reduces mitogenic activity and should be avoided. Once thawed, the medium can be aliquoted into smaller volumes and frozen for future use.

Shelf life is 12 months.

Use before expiry date given on the product label.

*Colcemid is a registered trademark of Ciba-Giegy Corp.*

IVD

In vitro diagnostic medical device

CE Marking



Consult instructions for use



Do not use if package is damaged



Reg. Off : 23, Vadhani Ind Est.,  
LBS Marg, Mumbai-400086, India.  
Works : B-4-5-6 / MIDC, Palkhed,  
Dindori, Nashik- 422202  
Maharashtra, India  
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EC REP

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Single use.  
Not intended to be  
reprocessed and/or used on  
another patient

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