

## HiMarrowXL™ Karyotyping Medium

With L-Glutamine, FBS, Gentamicin sulphate, Conditioned medium and Sodium bicarbonate  
1X Liquid Karyotyping Medium

**Product Code: AL301A**

### Intended Use:

HiMarrowXL™ Karyotyping Medium is a karyotyping medium recommended for short term *in vitro* culture of primary bone marrow cells for cytogenetic studies.

### 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.

AL301A is HiMarrowXL™ Karyotyping Medium composed of a basal medium and supplemented with L-Glutamine, FBS, gentamicin sulphate, conditioned medium and Sodium bicarbonate. It is a complete medium and does not require supplementation with any additional component.

### Type of Specimen:

Clinical samples – Bone marrow

### 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:

1. Add 250µl freshly collected bone marrow to 5ml of HiMarrowXL™ Medium in a sterile 15ml conical bottom centrifuge tube.
2. Loosen the cap of tube by one thread and incubate at 37°C and 5% CO<sub>2</sub> for 24-48 hours in horizontal position as shown in the figure below.

*Note: Alternatively, the tubes can be incubated in a non-CO<sub>2</sub> incubator. Absence of CO<sub>2</sub> does not affect the mitotic count.*

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

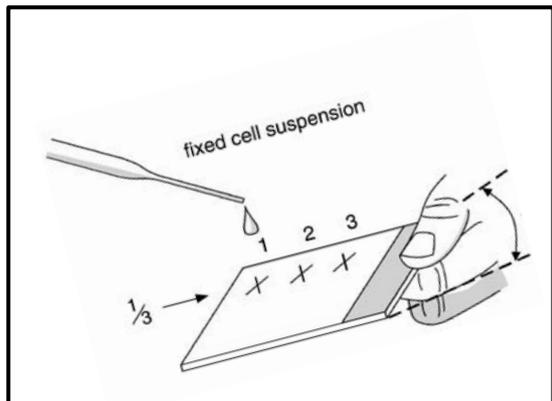
*Note: Incubation of 2 hours gives higher mitotic count than 20 minutes. Users are advised to decide incubation time as per their need and convenience.*

4. After incubation with colchicine, centrifuge the tubes at 1000rpm for 10 minutes.
5. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
6. 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.
7. Centrifuge the tubes at 1000rpm for 10 minutes.
8. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
9. 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.*

10. Repeat steps 7, 8 and 9 two more times.
11. Resuspend the pellet in 0.5ml of fresh fixative and store them at -20°C till slide preparation.
12. Clean the slides with mild detergent and wash thoroughly under tap water to make them grease free.
13. 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.*

14. Mix the cell suspension gently by pipetting up and down. DO NOT vortex.
15. 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.



18. Heat fix the slides by holding them over a hot plate for 10 – 12 seconds, with chromosome spreads facing up.
19. 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 H2O

340.00 -380.00

#### Sterility

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

#### Cultural Response

The growth promotion capacity of the medium is assessed qualitatively by analyzing the cells for the morphology and quantitatively by counting the metaphases.

### 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  
[www.himedialabs.com](http://www.himedialabs.com)

EC REP

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 DB Maarn The Netherlands,  
[www.cepartner4u.eu](http://www.cepartner4u.eu)



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

Revision: 02/2020

### Disclaimer:

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