HiMedia CellCulture
Enabling Breakthroughs

Product Information

HiKaryoXL™ Colcemid® Solution

w/ 10µg per ml Colcemid in Phosphate Buffered Saline

Product Code: TCL133

Product Description :

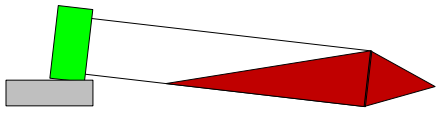
Colcemid®, also known as demecolcine, is a synthetic analog of colchicine. It is less toxic to cells than colchicine. Being a mitotic inhibitor, colcemid binds to the tubulin protein and obstructs the spindle fibre formation.
At very low concentration, it binds to microtubule plus end and affects the microtubule dynamics by causing depolymerisation. It effectively arrests cells in metaphase, allowing cell harvest and karyotyping to be performed. Colcemid is used to arrest different types of cells in metaphase like peripheral blood cells, amniotic fluid cells, fibroblasts, bone marrow cells and cells from chorion villus samples, etc.
Exposure of cells to Colcemid depends upon quantity of colcemid and the type of cell. A longer exposure will result in more metaphases but with shorter chromosomes whereas for cytogenetic studies, longer chromosomes are generally preferred

TCL133 is a sterile filtered solution of 10µg per ml of Colcemid in Phosphate Buffered Saline

Directions :

For Peripheral Blood Culture

1. Add 500µl freshly collected heparinized whole blood to 5ml of HiKaryoXL™ 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_2 for 70 - 72 hours in horizontal position as shown in the figure below:

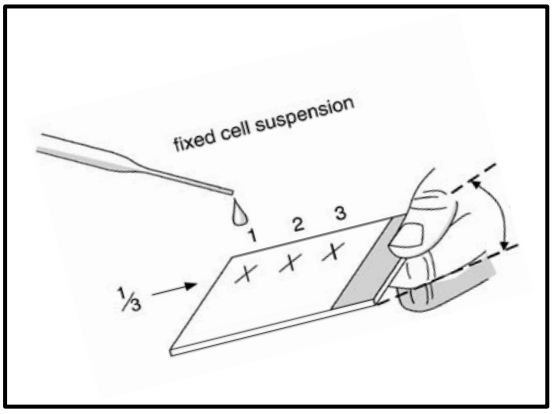


Note: Alternatively, the tubes can be incubated in a non-CO_2 incubator. Absence of CO_2 does not affect the mitotic count.

3. Add 250µl of 10µg/ml of Colcemid (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 2000rpm for 4 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 7 minutes keeping the tubes in an upright position. Mix by inverting.
7. Centrifuge the tubes at 2000rpm for 4 minutes.
8. Discard the supernatant and vortex briefly for 5 seconds to disperse the pellet uniformly.
9. Add 5ml 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:



16. Similarly drop 50µl suspension the center and 50µl at the top of the slide.
    Note: Ensure that the direction of dropping is from bottom to the top.
17. Allow the slides to air dry. DO NOT blow.
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™ RPMI Medium (AL165A) or
HiKaryoXL™ Nutrient Mixture F-10 Ham Medium (AL169A)
Potassium Chloride solution 0.075M (TCL040)
Methanol
Acetic Acid
Giemsa Stain (TCL083)

Quality Control:

Appearance
Colorless, clear solution

pH
7.00 - 7.60

Osmolality in mOsm/Kg H_2O
280.00 - 320.00

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.
Shelf life of the product is 6 months.
Use before expiry date given on the product label.

Revision : 3 / 2019