HiKaryoXL™ Colcemid Solution

With 10µg per ml Colcemid in Hank's Balanced Salt Solution

Product Code: TCL074

Product Description :

Colcemid, also known as demecolcine, is a synthetic analog of colchicine. It is less toxic to cells than colchicine. Being a mitotic inbitor, 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 microubule dynamics by causing depolymerisation. It effectively arrests cell 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. Longer chromosomes are generally preferred for cytogenetic studies.

TCL074 is a sterile filtered solution of 10µg per ml of Colcemid is Hank's balanced salt solution.

Directions :

For Peripheral Blood Culture

1. Add whole blood to 10ml of HiKaryoXLTM Medium in T-25cm2 flasks as per the following recommendations:
- Normal adults - 0.8ml
- Infants and children- 0.6ml
- Women (during pregnancy/ postpartum) - 1.0ml

2. Incubate the flasks at 37°C and 5% CO2 for 67-72 hours.
3. To determine optimum incubation time i.e. the peak mitotic index, collect samples at different time intervals between 48-72 hours.
Note: Peak mitotic index is most commonly observed at 67-72 hrs.
4. Add 100µl of 10µg/ml of colcemid (TCL074) and incubate for additional 2 hours.
Note: Incubation time of less than 1 hour might result in reduced mitotic index.
5. Transfer the entire content of the flask to a sterile centrifuge tube and centrifuge at 800-1000rpm for 10 minutes.
6. Discard the supernatant and resuspend the pellet in 5ml of hypotonic 0.075M KCl solution and incubate in a water bath at 37°C for 15-20 minutes.
Note: Add KCl solution drop wise while agitating the cells
7. Add equal amount of freshly prepared ice cold fixative (Acetic acid: methanol, 1:3 parts)
8. Centrifuge cells at 800-1000rpm for 10min.
9. Discard the supernatant and again add 5ml of freshly prepared ice-cold fixative (acetic acid: methanol, 1:3 parts) with constant mixing. Leave the cells at 4°C for 10-15 min.
10. Centrifuge the cells at 1000rpm for 10 minutes.
11. Repeat step no. 9 and 10.
12. Discard the supernatant and resuspend the pellet in 0.2ml of fresh fixative.
13. Put 1 drop of cell suspension on to a clean, cold slide. Tilt the slide and let the drop run down the slide as it spreads and dry the slide rapidly over a hot plate or a beaker of boiling water.
14. Stain the slides as required.

Material 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 H2O
300.00 -340.00

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

Performance test
Complies

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.

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