

HiKaryoXL™ Colchicine Solution

With 10µg per ml Colchicine in phosphate buffered saline

Product Code: TCL062

Product Description :

Colchicine is an alkaloid that is isolated from the plant *Colchicum autumnale*. Colchicine is used for arresting the cells in metaphase. Arresting of cells in metaphase allows an increased yield of mitotic cells for analysis. Colchicine inhibits microtubule polymerisation by binding to tubulin. Availability of tubulin is essential for mitosis and therefore colchicine effectively arrest cells in metaphase.

TCL062 is a sterile filtered solution of 10µg per ml Colchicine in phosphate buffered saline.

Directions :

For Peripheral Blood Culture

1. Add whole blood to 10ml of HiKaryoXL™ 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 colchicine 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.

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 2-8°C.

Shelf life of the product is 6 months.

Use before expiry date given on the product label.

Revision : 1 / 2011

Disclaimer :

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