Datasheet

Demecolcine

Media Supplement

Product	Description	Catalogue-No.	Size
Demecolcine	**Microtubule depolymerizing drug solution (10 µg/ml)**	**P07-91010** **P07-91050**	**10 ml** **50 ml**

Product description

Demecolcine (N-deacetyl-N-methylcolchicine) also known as colcemid is an alkaloid from *Colchicum autumnale* and it is closely related to colchicine with the replacement of the acetyl group on the amino moiety with methyl and for humans it is 40 times less toxic. Demecolcine depolymerizes microtubules. Thereby it limits microtubules formation and inactivates the spindle fiber mechanism during metaphase. Colcemid binds tubulin rapidly in comparison to colchicine which binds tubulin relatively slowly.

Demecolcine is used for lymphocyte karyotyping, amniotic fluid cell and cancer cells chromosome analysis. By preventing spindle formation during mitosis, it causes metaphase arrest. Metaphase is the optimum phase of mitosis for microscopically visualizing the chromosomes. By treating cells with a hypotonic solution and fixation (methanol : glacial acetic acid), metaphase chromosomes can be microscopically observed and analyzed.

Demecolcine is used to increase the percentage of metaphase cells for chromosome analysis.

Storage conditions

Storage: -20°C, in the dark, keep tightly closed
Stability: 2 years from date of production
Size: 10ml, 50ml, other sizes on request

Composition

10 µg/ml Demecolcine ($C_{21}H_{25}NO_5$) in DPBS buffer. It is filtered through 0.2µm pore size membrane.

Caution

Demecolcine solution is mutagenic, tumorigenic, embryotoxic and teratogenic.

Repeated thawing and freezing of demecolcine damages the biological activity.
Hence, demecolcine can be aliquoted and frozen only for one time.
Stock solutions are stable for up to 4 weeks at +4 to +8°C.
Keep sterile and do not use if a visible precipitate is observed in the solution.
Suggested working concentration is 0.1-0.5µg/ml.

Suitability

Demecolcine is suitable for a variety of adherent and non-adherent cells. For scientific research in cells demecolcine is mostly used for the study of mitosis, like chromosomes-movement during the M-phase, cell migration and cell division. It is also used for cell synchronization or for chromosome analysis at metaphase-stage. Metaphase-Analysis in cytogenetic like karyotyping (e.g. amniotic fluid cell, Bone Marrow, Hematopoietic Cell,) by arresting them at metaphase.

Demecolcine, depending on dose, has also been found to cause aneuploidy, nondisjunction of chromosomes micronuclei.

Medically demecolcine has been used to improve the results of cancer radiotherapy by synchronising tumour cells at metaphase, the radiosensitive stage of the cell cycle.

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Metaphase Spread

Metaphase Spread is an analysis technique of chromosomes arrested during metaphase. At this stage of cell division the chromosomes are highly condensed and are visible under a light microscope.

Prepare:

- Fixative solution (methanol:glacial acetic acid 3:1), keep it at -20°C (at least 1 hour or overnight).
- KCl solution 75 mM KCl (prewarmed to 37 ºC).

Suggested working concentration is 0.1-0.5µg/ml. It is depending on the cell type and the cell density.

1. Add Demecolcine to your cell culture with a final concentration of ~ 150ng/ml for 30 min – 4 hrs.
2. Collect cells and transfer them into a tube.
3. Spin cells down at ~ 200 x g for 10 min.
4. Aspirate supernatant, leave ~ 0.2-0.5 ml of media and resuspend cells by tapping bottom of tube.
5. Add 1 ml of 75 mM KCl (prewarmed to 37 ºC) dropwise, gently mix cells with pipette.
   Add 9 ml of 75 mM KCl (prewarmed to 37 ºC) dropwise.
6. Incubate 16-20 min at 37 ºC (incubator or water bath).
7. Add 4 ml of ice-cooled fixative, invert tube, wait 1 min and spin down cells at ~ 200 x g for 10 min.
   If you have no or a very petty cell pellet, spin down cells again at ~ 220 x g for 10 min.
8. Aspirate supernatant leaving 0.5 ml.
9. Resuspend pellet by tapping and add 5 ml ice-cooled fixative dropwise for the first 1 ml while gently vortexing. Incubate ~ 15 min at 4 ºC. Spin cells down at ~ 200 x g for 10 min.
10. Repeat step 9 two - three times.
11. Resuspend the cell pellet in a small volume of fixative.
12. The cells are ready for further investigations or keep them at -20 °C up to 6 months.

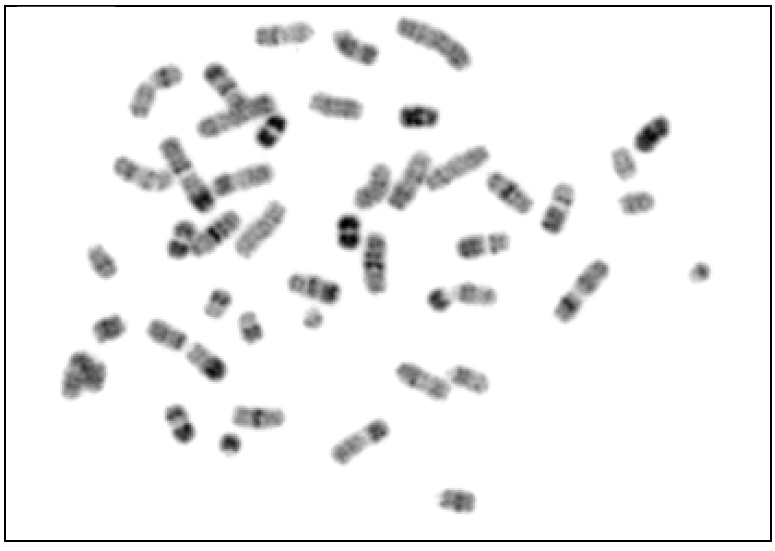

Fig.1 Fluoreszenz-in-situ-Hybridisierung of *Alu*-sequences human (XX) metaphase spreads

Technical support

For technical support, questions or remarks please contact your local PAN-Biotech partner or the technical department of PAN-Biotech via email (info@pan-biotech.com) or phone +49-8543-601630.

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