ClearLine	Technical data sheet	Ref : 509033
		Version date : 18/07/2022

Colcemid 10µg/ml in PBS (Demecolcin)

Storage conditions: +2°C to +8°C

Shelf life: 24 months

Composition: Displayed on website and in catalogue; also available on request

Colour: Colourless, clear solution

pH: 7.2 ± 0.3

Osmolality: 295 mOsm/kg ± 10 %

Endotoxin: < 10 EU/ml

Sterility tests:
- Bacteria in aerobic and anaerobic conditions
- Fungi and yeasts

Cell Growth test: Not applicable

Other tests: Not applicable

Recommended use:
- Respect storage conditions of the product
- Do not use the product after its expiry date
- Store product in an area protected from light (not necessary for saline solutions).
- Manipulate the product in aseptic conditions (e.g.: under laminar air flow)
- Wear clothes adapted to the manipulation of the product to avoid contamination (e.g.: gloves, mask, hygiene cap, overall…)

The product is intended to be used in vitro for research or further manufacturing only and not for use as an Active Pharmaceutical Ingredient or food or animal feed.

Application:
One application of Colcemid solution is: Harvesting Cultured Lymphocytes for In-Situ Hybridization Metaphases
There are 3 steps to the harvesting procedure:

- Dividing cells are arrested in mitosis with the use of colcemid. Colcemid prevents the formation of the spindle apparatus responsible for cell division, thereby permitting an accumulation of metaphases. Note: Lymphoblastoid cell lines can also be prepared for chromosome analysis, because the morphology of lymphoblastoid cells is comparable to lymphocytes prepared from whole blood samples.

- The addition of the hypotonic solution, potassium chloride (KCl), will result in water rushing into the cells due to the concentration gradient caused by the less-than-physiological concentration outside of the cells. The cells will swell and cytoplasmic membranes will stretch. The cells resemble a water balloon with chromosomes suspended inside.
- With the addition of fixative (1:3: methanol:acetic acid), each cell is preserved in its swollen shape. Chemical changes make the cell harden (not as fragile as in hypotonic), but remaining a swollen sac of suspended chromosomes.

Time required:
2-3 hours when harvesting 8-12 culture tubes on day 4 of chromosome preparation (on days 1-3, the lymphocytes are stimulated to divide in culture media).

Procedure:

1. On day 4, or 72 hours after the culture was initiated, prewarm 0.075 M KCl at 37°C and chill a 500 ml bottle of methanol to 4°C.
2. After 72 hours of culture incubation, add 0.25 ml colcemid (10 µg/ml) with a 1cc syringe to each 15 ml tube. Invert tubes several times to mix. Return cultures to the 37°C incubator for 10-20 minutes. Note: The length of chromosomes is dependent on the concentration of colcemid and the time of mitotic arrest. The higher the concentration of colcemid and the longer the time of mitotic arrest, the shorter the chromosomes will be. Therefore, it is best to keep the concentration of colcemid and the time of exposure to a minimum. Colcemid will continue to be active until the addition of fixative. Phytohemagglutinin (PHA) stimulated cultures require a very short time of exposure (10-30 minutes) to colcemid because they are rapidly-dividing cells.
3. Resuspend the cells again by inverting the tubes and centrifuge cultures for 8 minutes at 1200 rpm in the Beckman TJ-6 centrifuge (for exemple).
4. Aspirate the supernatant with a sterile pasteur pipet connected to a vacuum apparatus. Resuspend the cells with a pasteur pipet. Slowly add 1-3 ml of warm hypotonic solution. Continue to add 1-3 ml little by little, until 10 ml of KCl has been added. Blow air into the suspension with a pipet after each addition of KCl solution. Incubate the tubes for 18 minutes at 37°C.
5. Add 1 ml of freshly prepared cold fixative (1:3: methanol, 4°C: acetic acid) to each culture **very slowly** (drop by drop). Bubble air through the suspension after every drop. This will quickly fix the cells in a swollen shape and begin lysing red blood cells.
6. Centrifuge the cells for 8 minutes at 1200 rpm in the TJ-6 centrifuge (for exemple).
7. Aspirate the supernatant (contains lysed red blood cells and cellular debris). Resuspend the cell suspension by bubbling air through the pellet and add 1-3 ml of fixative. Continue washing cells with fixative, until a total of 10 ml of fixative has been added.
8. Repeat step 6 and step 7, three more times or until all red cells are lysed and a white cell pellet is observed.
9. Drop the harvested metaphases to the absolute ethanol rinsed microslides (refer to microslide preparation procedure). For best results, this step should be done immediately after the metaphases cells are harvested. If microslides cannot be

prepared immediately, the cell suspensions can be stored in the fixative solution at 4°C for 24 hours.

Solutions:
Prepare fixative by mixing 75 ml of 4°C methanol with 25 ml glacial acetic acid. Fixative should be made fresh before each use, because it can absorb water upon standing and the pH changes with time.

References:
Monteleone, P., Department of Genetics, Cardinal Glennon Children's Hospital. April 24, 1990
The association of Cytogenetic Technologists Cytogenetic laboratory manual; University of California, San Francisco, California 94143
Yunis, J.J., and Chandler M.E., Cytogenetics, chapter 26

Uses: Not applicable

Signs of Deterioration: Not applicable

Remarks: Not applicable