Lymphocyte Synchronization Kit
For high-resolution cytogenetic analysis
For research use only

Catalogue number: BI-2002

Product Description

The blood cell karyotyping method was developed to provide information about chromosomal abnormalities. Lymphocyte cells do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

High resolution analysis is a special manipulation of the routine blood karyotyping procedure designed to provide a large number of mitotic figures in late prophase or prometaphase. At this stage of mitosis the chromosomes are longer and less condensed. After G-banding, the chromosomes will show greater level of band resolution not seen in routine analysis. High resolution allows more detailed analysis of the karyotype. Cultures can be synchronized by the addition of methotrexate (MTX), an inhibitor of thymidine biosynthesis which blocks cells in the S-phase (DNA synthesis) of the cell cycle. After 16-18 hours, most of the dividing cells in the culture are in the S-phase. If thymidine is added to the culture, the MTX block is released and the cells proceed syn
to mitosis, at which point colchicine may be added. A very short colchicine treatment in conjunction with this technique may be used to produce extended prometaphase chromosomes when small deletions or rearrangements are suspected.

Ingredients

1. LymphoPlus (BI-1103, optimized complete medium for lymphocyte culture), one 100ml bottle
2. MTX, 10-5 M in HBSS: 1 vial containing 1.5 ml each.
3. Thymidine, 10-3 M in distilled water: 1 vial containing 1.5 ml each.

How to Use

1. Defreeze LymphoPlus medium (4ºC, overnight).
2. Inoculate approximately 0.5 ml of fresh heparinized whole blood into a glass or plastic tube with 3-5 ml of LymphoPlus medium. Don’t use EDTA for heparinization.
3. Incubate the culture for 72 hours (check CO2 and temperature of the incubator).
4. After 48 hours, add 0.1ml of MTX Solution per tube and carefully agitate. It is recommended to add the MTX in the afternoon - for overnight incubation (17 hours). Since MTX is toxic, do not extend the incubation time.
5. After 17 hours, add 0.1ml of Thymidine solution per tube with continuous vortexing. Incubate for 4-5.5 hours. Please note that duration of G2 stage for lymphocytes is only 4-5.5 hours. Therefore, if you extend the incubation time, cells will pass metaphase and you will miss mitotic cells.
6. After 4-5.5 hours, add 0.1ml of colchicine Solution to each culture tube. If long pro-metaphase chromosomes are desired, harvest after 10-20 minutes. If a high mitotic index is desired, harvest after 30-50 minutes of incubation.

7. Transfer the culture to a centrifuge tube and spin at 500g for 5 minutes.
8. Remove the supernatant and re-suspend the cells in 5-10ml of hypotonic 0.075M KCl. Incubate at 37ºC for 10-12 minutes.
9 . Spin at 500g for 5 minutes.
10. Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5-10ml of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol. Leave in 4ºC for 10 minutes.
11. Repeat steps 9 and 10.
12. Re-suspend the cell pellet in a small volume 0.5-1ml of fresh fixative, drop onto a clean slide and allow to air dry.
13. At this stage, the preparation can be stained.

Inhibitors

- Metal chelating agents such as cysteine, EDTA or o-phenanthroline but not di-isopropyl fluorophosphate (DFP). It is also inhibited by a -macroglobulin, a large plasma glycoprotein. 2

NOTE

- The solutions must be kept frozen and protected from light. If appropriately stored, the solutions are stable for at least 18 months from the date of preparation

Warning!

- Methotrexate has caused adverse reproductive and foetal effects in humans. May cause eye, skin, and respiratory tract irritation.
- May cause blood abnormalities. May cause heritable genetic damage.

References

1. Kelly, T.E., 1986. Clinical genetics and genetic counselling.
2. Knutsen, T., 1992. International Cytogenetic Laboratory Directory. Association of Cytogenetie Technologists, Burbank, California.
3. Moorhead, P.S., Nowell, P.C., Mellman, W.J., Battips, D.T. and Hungerford, D.A., 1960. Chromosome preparations of leukocytes cultured from human peripheral blood. Experimental cell research, 20(3), pp.613-616.
4. Barch, M.J., The association of cytogenetic technologists' laboratory manual. New York: Act.
5. Nowell, P.C., 1960. Phytohemagglutinin: an initiator of mitosis in cultures of normal human leukocytes. Cancer research, 20(4), pp.462-466.