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Biological Industries

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Your Molecular & Cell Technology Partner

Cytogenetics

Cell Synchronization Kit

For high-resolution cytogenetic analysis

Cat. No.: 12-008-60
Store at: -20°C

Instructions for Use

Principle

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 synchronously to mitosis, at which point colcemid may be added. A very short colcemid treatment in conjuction with this technique may be used to produce extended prometaphase chromosomes when small deletions or rearrangements are suspected.

Materials

1. Methotrexate (Amethopterin), 10^{-5}M in HBSS: 4 vials containing 1.5ml each
2. Thymidine, 10^{-3}M in distilled water: 4 vials containing 1.5ml each

Storage and Stability

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.

Procedure

1. Set up the blood culture according to the specific medium instructions **(Cat. No.: 01-198-1 , 01-201-1).**
2. Inoculate approximately 0.5ml of heparinized whole blood into a glass or plastic tube with 10ml of medium.
3. Incubate the culture for 72 hours.
4. After 48 hours add (with carefull agitation), MTX Solution to a final concentration of 10^{-7}M (0.1ml from 10^{-5}M stock solution per tube). It is recommended to add the MTX in the afternoon - for overnight.
5. After 17 hours, add (with continous vortexing) Thymidine Solution to a final concentration of 10^{-5}M (0.1ml from 10^{-3}M stock solution per tube).
6. After 5-5.5 hours, add 0.1ml of **Colcemid Solution (Cat. No. 12-004-1)** to each culture tube. **If long, prometaphase 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 (Cat. No. 12-005-1)**. 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. Spin at 500g for 5 minutes.
13. 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.
14. At this stage, the preparation can be stained with Orecin or Giemsa. Giemsa banding has become the most widely used technique. The most common method to obtain this staining is to treat slides with **Trypsin-EDTA 10X (Cat. No. 03-051-5).**

Related Products

Product	Cat. No.
PB Karyotyping Medium	01-198-1
Bone Marrow Karyotyping Medium	01-199-1
Hematopoietic Cell Karyotyping Medium	01-200-1
PB Karyotyping Medium with PHA-M	01-201-1
Trypsin EDTA, 10X concentrate	03-051-5
Colcemid Solution	12-004-1
0.075M KCl Solution	12-005-1
PHA-M	12-009-1

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