Cell Synchronization Kit product information

Product Name

Name: Cell Synchronization Kit

Cat. No.: C3610-0012

Size: Solution-A (1.5 mL* 4 vials) + Solution-B(1.5 mL* 4 vials)

Product Description

One of the most common methods to visualize chromosomal abnormalities is karyotyping, which examines the gross chromosome pairing and ordering from mitotic cells. Karyotypes can reveal gross chromosomal changes such as aneuploidy in Down syndrome, or more subtle structural changes such as chromosomal deletions, duplication, translocations, or inversions. Blood and bone marrow cell karyotyping has been developed to provide information on chromosomal abnormalities. Lymphocytes in the blood or lymphatic tissues do not normally undergo cell divisions unless they are activated by mitogens. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis. After 48-72 hours, a mitotic inhibitor is added to the culture to stop mitosis in metaphase. After treatment by hypotonic solution, fixation, and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

To obtain more precise karyotypic analysis, highresolution G-banding analysis can provide a large number of mitotic figures in late prophase or prometaphase following cell synchronization. At this stage of mitosis, the chromosomes are longer and less condensed. After G-banding, the chromosomes will show a greater level of band resolution not seen in routine analysis. Cultures of cells can be synchronized by the addition of methotrexate (MTX), an inhibitor of thymidine biosynthesis, to block the cells in S-phase (DNA synthesis) of the cell cycle. After 16-18 hours, most of the dividing cells in the culture are in S-phase. Thymidine is then added to the culture to release the MTX block, and the cells proceed synchronously to mitosis, at which point colcemid is added. A brief colcemid treatment in conjunction with the use of the cell synchronization

kit can produce karyotypes with extended prometaphase chromosomes when small deletions or rearrangements are suspected.

Materials

1. Solution-A: Methotrexate (MTX, or Ametho-pterin), 10^{-5} M: 4 vials containing 1.5 mL each.
2. Solution-B: Thymidine (T), 10^{-3} M: 4 vials containing 1.5 mL each.

Procedure

Example: Blood cell karyotyping

1. Set up the blood culture according to the specific medium instructions used in your laboratory.
2. Inoculate approximately 0.5 mL of heparinized whole blood into a glass or plastic tube with 10 mL of medium.
3. After 48 hours, add Solution-A with careful agitation to a final concentration of 10^{-7} M (0.1 mL from 10^{-5} M stock solution per tube or 1:100 dilution).
4. After 16-18 hours, add Solution-B with continuous vortexing to a final concentration of 10^{-5} M (0.1 mL from 10^{-3} M stock solution per tube or 1:100 dilution).
5. After 5-6 hours, add an appropriate amount of colcemid solution (VivaCell, Cat. #C3541) to each culture tube. If long, prometaphase chromosomes are desired, harvest after 10-20 minutes. If a high mitotic index is desired, then harvest after 30-50 minutes of incubation. Forty-five minutes are recommended for the first trial.

6. Transfer the culture to a centrifuge tube and spin at 500 x*g* for 5 minutes.
7. Remove the supernatant and re-suspend the cells in 5-10 mL of hypotonic 0.075 M KCl (VivaCell, Cat. #C3540) preheated to 37ºC.
8. Incubate at 37 ℃ for 10-12 minutes.
9. Spin at 500 x*g* for 5 minutes.
10. Remove the supernatant, agitate the cellular sediment, and add drop-by-drop 5-10 mL of fresh, fixative at 4 ℃ (made up of 1 part acetic acid to 3 parts methanol). Leave at 4 ℃ for 10 minutes.
11. Repeat steps 9 and 10.
12. Spin at 500 x*g* for 5 minutes.
13. Resuspend the cell pellet in a small volume (0.5-1 mL) of fresh fixative (this volume can be used to adjust the density of the cells on the slides).
14. Drop the cells onto cleaned slides and allow to air dry.

At this stage, the preparation can be treated with trypsin solution and stained with Giemsa solution for G-Banding karyotyping.

Storage and Stability

The product should be kept at **-20°C**.

The product must be kept **frozen and protected from light.**

If appropriately stored, the solutions are stable for at least 18 months from the date of manufacture.

Precautions and Warning

- Methotrexate will cause adverse reproductive and fetal effects in humans.
- It may cause eye, skin, and respiratory tract irritation.
- It may cause blood abnormalities.
- It may cause heritable genetic damage.

Quality Control

Cell Synchronization Kit is tested for sterility, cell culture. For full specifications please check the lot-specific Certificate of Analysis (CoA).

Quality Assurance

- Manufactured under ISO 13485 QMS and in compliance with applicable cGMP guidelines.
- Manufactured under controlled environments and processes in accordance with:
  1. ISO 13408 – Aseptic Processing of Health Care Products;
  2. ISO 14644 – Airborne Particulate Cleanliness Classes in Clean Rooms and Clean Zones.

Manufacturer

Shanghai Dr. Cell Co., Ltd.

Issue Date

January 2025

Precaution and Disclaimer

For research use only, not for clinical diagnosis, and treatment.