Cell Synchronization Kit product information

PI-C3611 V1.1

Product Name

Name: Cell Synchronization Kit, Enhanced

Cat. No.: C3611-0009

Size: Solution A (0.7 mL* 4 vials) + Solution B (1.5 mL* 4 vials)

Product Description

Karyotyping is one of the most widely used cytogenetic techniques for detecting chromosomal abnormalities. It involves the microscopic examination of chromosome number and structure, typically using metaphase chromosomes derived from mitotic cells. Karyotypic analysis can identify both numerical abnormalities, such as aneuploidy seen in conditions like Down syndrome, and structural aberrations, including deletions, duplications, translocations, and inversions.

In clinical and research settings, karyotyping is commonly performed on dividing cells from peripheral blood or bone marrow. Under normal physiological conditions, lymphocytes—the primary cell type used in blood karyotyping—remain in a quiescent, non-dividing state. However, when stimulated with mitogens (e.g., phytohemagglutinin), lymphocytes are induced to enter the cell cycle and undergo mitosis. After an incubation period of 48–72 hours, a mitotic inhibitor such as colcemid is added to arrest cells in metaphase, the stage where chromosomes are most condensed and visible. Following treatment with a hypotonic solution, fixation, and staining (e.g., Giemsa), chromosomes are spread on glass slides and visualized microscopically for analysis.

To enhance the resolution and diagnostic precision of karyotypic analysis, high-resolution G-banding can be employed. This method captures chromosomes in late prophase or prometaphase, when they are longer and less condensed, allowing for the detection of subtle chromosomal alterations that may not be visible in standard metaphase preparations. Achieving this higher resolution involves cell cycle synchronization, often using methotrexate (MTX), a folate antagonist that inhibits thymidine synthesis and arrests cells in the S phase of the cell cycle.

After approximately 16–18 hours, most actively dividing cells are synchronized in S phase. Thymidine is then added to relieve the MTX block, allowing the cells to progress synchronously into mitosis. To capture chromosomes at the optimal stage for high-resolution analysis, colcemid is briefly applied after release from synchronization. This process results in a population of cells arrested in extended prometaphase, suitable for detecting small deletions, duplications, or complex rearrangements. The use of a cell synchronization kit in conjunction with carefully timed colcemid treatment significantly improves the yield and clarity of mitotic spreads, enhancing diagnostic capability in both constitutional and acquired chromosomal disorders.

Materials in the kit

1. Solution A: Methotrexate (MTX, or Amethopterin), 10^{-4} M: 4 vials each containing 0.7 mL.
2. Solution B: Thymidine (T), 10^{-3} M: 4 vials each containing 1.5 mL.

Procedure

Example: Blood cell karyotyping

1. Initiate the blood culture according to the specific medium instructions provided by your laboratory protocol or supplier guidelines.
2. Inoculate approximately 0.5 mL of heparinized whole blood into a sterile glass or plastic culture tube containing 5 mL of culture medium.
3. After 48 hours of incubation, add Solution A to each culture tube. Gently agitate to mix and achieve a final concentration of 1×10^{-7} M (equivalent to adding 5 μL from a 1×10^{-4} M stock solution, i.e., a 1:1000 dilution).

4. After an additional 16-18 hours, add **Solution B** to each tube with continuous vortexing, reaching a final concentration of 1 × 10^{-5} M (by adding 50 μL from a 1 × 10^{-3} M stock solution, a 1:100 dilution).
5. Following 5-6 hours of further incubation, add an appropriate volume 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 cultures to centrifuge tubes and centrifuge at 200 × *g* for 5 minutes.
7. Carefully remove the supernatant, and resuspend the cell pellet in 5-10 mL of 0.075 M KCl hypotonic solution (VivaCell, Cat. #C3540), pre-warmed to 37 °C.
8. Incubate the cell suspension at 37°C for 15-30 minutes to induce cellular swelling.
9. Centrifuge again at 200 × *g* for 5 minutes.
10. Discard the supernatant. Gently agitate the cell pellet and add 5-10 mL of freshly prepared fixative (3:1 methanol: acetic acid) dropwise at 4 °C. Allow the suspension to stand at 4°C for 10 minutes.
11. Repeat steps 9 and 10 to ensure thorough fixation.
12. Centrifuge once more at 200 × *g* for 5 minutes.
13. Resuspend the final cell pellet in a minimal volume (0.5-1.0 mL) of fresh, cold fixative. Adjust this volume based on the desired cell density for slide preparation.
14. Prepare chromosome spreads by dropping the fixed cell suspension onto **clean, grease-free** microscope slides, which can be cleaned by rinsing them in methanol and wiping with lint-free paper. Allow the slides to air dry completely.
15. At this stage, slides may be treated with trypsin (VivaCell, Cat. #C3683) followed by Giemsa (VivaCell, Cat. #C3720) staining to perform G-banding for karyotypic analysis.

Storage and Stability

- The product should be kept at **-20°C~-10°C**.
- The product must be kept **frozen and protected from light. Avoid repeated freezing and thawing.**
- 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 and cell culture performance. 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

June 2025

Revision Date: February 2nd, 2026

Precaution and Disclaimer

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