



BIO-HEMATO™ Karyotyping Medium

With conditioned medium
for bone marrow
and peripheral blood
hematopoietic cells

Cat. No.: 01-200-1

Store at: -20°C

Instructions for Use

Product Description

Cytogenetic analysis of human hematopoietic cells using bone marrow aspirates is a standard practice in hematology. Fresh cells or cells grown in short-term cultures often yield an insufficient number of mitotic cells and repeated aspirations are required. BIO-HEMATO™ Karyotyping Medium was developed to stimulate the proliferation of human hematopoietic cells from bone marrow as well as peripheral blood. This medium is particularly effective for karyotyping of acute non-lymphocytic leukemias and various stages of chronic myelogenous leukemia as well as other hematological disorders such as myelodysplastic syndrome and polycythemia vera. BIO-HEMATO™ Karyotyping Medium is based on MEM-Alpha basal medium supplemented with L-Glutamine, foetal bovine serum, antibiotics (gentamicin) and conditioned medium.

BIO-HEMATO™ Karyotyping Medium is supplied as frozen medium, which is ready for use after thawing.

Precaution and Disclaimer

- For *in vitro* diagnostic use. The medium is not intended for therapeutic use.
- Do not use if a visible precipitate is observed in the medium.
- Use of Biological Industries BIO-HEMATO™ Karyotyping Medium does not guarantee the successful outcome of any chromosome analysis testing.
- Do not use BIO-HEMATO™ Karyotyping Medium beyond the expiration date indicated on the product label.

Storage and Stability

BIO-HEMATO™ Karyotyping Medium should be kept frozen at -20°C.

After thawing, the medium should be stored at 2-8°C.

The medium should be used within 10 days after thawing.

Protect the medium from light.

Instructions for Use

Thaw BIO-HEMATO™ Karyotyping Medium at refrigerator temperatures (2-8°C) or at room temperature. Mix gently after thawing.

Note that the medium already contains L-Glutamine.

Culture of Bone Marrow and Peripheral Blood Cells for Chromosome Analysis

The hematopoietic cell karyotyping method was developed to provide information about chromosomal abnormalities. In the presence of a conditioned medium, acute and chronic nonlymphocytic leukemic cells in bone marrow and peripheral blood cultures 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.

Test Procedure

1. Inoculate approximately 0.5ml of bone marrow suspension or $0.5-1 \times 10^7$ Ficoll-separated peripheral blood cells into a plastic tube or tissue culture plate with 10ml of medium. Invert tubes gently to mix specimen.
2. Incubate the culture for 72-120 hours.
3. Add 0.1-0.2ml of **Colcemid Solution (Cat. No. 12-004-1)** to each culture tube. Incubate the culture for an additional 15-30 minutes.
4. Transfer the culture to a centrifuge tube and spin at 500g for 5 minutes.
5. Remove the supernatant and re-suspend the cells in 5-10ml of hypotonic **0.075M KCl (Cat. No. 12-005-1)**. Incubate at 37°C for 10-12 minutes.
6. Spin at 500g for 5 minutes.
7. 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 4°C for 10 minutes.
8. Repeat steps 6 and 7.
9. Spin at 500g for 5 minutes.
10. 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.
11. 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)**.

Quality Control

BIO-HEMATO™ Karyotyping Medium is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each batch is tested for leukemic cell growth in a leading clinical hematology laboratory.

Related Products

Product	Cat. No.
Trypsin EDTA, 10X concentrate	03-051-5
Colcemid Solution	12-004-1
0.075M KCl Solution	12-005-1
BIO-MARROW™ Karyotyping Medium	01-199-1



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