BI
Biological Industries
Culture of Excellence

Life Technologies™
Your Molecular & Cell Technology Partner

BIO-HEMATO™ Karyotyping Medium

With conditioned medium for bone marrow and peripheral blood hematopoietic cells

REF 01-200-1

-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

- 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 marrowsuspension or 0.5-1x10^7 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 anadditional 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 37ºC 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 4ºC 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 Orecinor 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 karyotyping 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

Quality Assurance

- For in vitro diagnostic use. The medium is not intended for therapeutic use.
- Listed in Europe under CE IVD class I, thus comply with European In-Vitro Diagnostic Devices Directive (98/79/EC) requirements.
- Manufactured under ISO 13485 QMS.
- Manufactured under controlled environments and processes in accordance with:
  1. ISO 13408 – Aseptic Processing of Health Care Products;
  2. ISO 14644 – Cleanrooms and associated controlled environments

EC REP Authorized Representative in the European Community:
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Product Label Symbols

REF	Indicates the manufacturer's catalogue number so that the product can be identified.
LOT	Indicates the manufacturer's batch code so that the batch or lot can be identified. Note: Synonyms for batch code are lot number and batch number.
	Indicates the date after which the product is not to be used.
	Indicates the temperature limits to which the product can be safely exposed.
STERILE A	Indicates a product that has been manufactured using accepted aseptic techniques.
CE	Indicates that the product meets the requirements of the applicable EC directives
IVD	Indicates a product that is intended to be used as an in vitro diagnostic medical device.
	Indicates the need for the user to consult the instructions for use.