



BI
Biological Industries

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Your Molecular & Cell Technology Partner

Bone Marrow Karyotyping Medium

without conditioned medium

Cat. No.: 01-199 -1
Store at: -20°C

Instructions for Use

Product Description

Biological Industries Bone Marrow Karyotyping Medium is intended for use in short-term cultivation of primary bone marrow cells for chromosome evaluation. Bone Marrow Karyotyping Medium is based on RPMI-1640 basal medium supplemented with L-Glutamine, foetal bovine serum, and antibiotics (gentamicin). The medium does not contain any mitogens or conditioned medium.

Bone Marrow 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 Bone Marrow Karyotyping Medium does not guarantee the successful outcome of any chromosome analysis testing.
- Do not use Bone Marrow Karyotyping Medium beyond the expiration date indicated on the product label.

Storage and Stability

Bone Marrow 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 Bone Marrow Karyotyping Medium at refrigerator temperatures (2-8°C) or at room temperature. Mix gently after thawing.

The medium may be supplemented with growth factors or mitogens if required.

Note that the medium already contains L-Glutamine.

Culture of Peripheral Blood Lymphocytes for Chromosome Analysis

The bone marrow karyotyping method was developed to provide information about chromosomal abnormalities. The ready-to-use medium is intended for the culture of bone marrow cells without any mitogens or conditioned medium. 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 into a plastic tube or tissue culture plate with 10ml of medium. Invert tubes gently to mix specimen.
2. Incubate the culture for 72 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. 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.
10. 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

Bone Marrow Karyotyping Medium is tested for sterility, pH, osmolality and endotoxin concentrations.

Related Products

| Product | Cat. No. |
|---------------------------------------|----------|
| Trypsin EDTA, 10X concentrate | 03-051-5 |
| Colcemid Solution | 12-004-1 |
| 0.075M KCl Solution | 12-005-1 |
| Hematopoietic Cell Karyotyping Medium | 01-200-1 |



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