ID Labs™ inc
Biotechnology

CE

www.idlabs.com
idinfo@idlabs.com

Karyotyping Medium – BM (without conditioned medium)

Catalog No.	IS1012 100 ml
Introduction	ID Labs' Karyotyping Medium - BM is intended for use in short-term cultivation of primary bone marrow cells for chromosome evaluation. Karyotyping Medium - BM is based on RPMI-1640 basal medium supplemented with L-Glutamine, fetal bovine serum, and antibiotics (penicillin and streptomycin). The medium does not contain any mitogens or conditioned medium.
Format	Karyotyping Medium - BM is supplied as frozen medium, which is ready for use after thawing.
Reconstitution	Thaw Karyotyping Medium - BM at refrigerator temperatures (2-8 ^{o}C) or at room temperature. Mix gently after thawing. The medium may be supplemented with growth factors or mitogens if required. The medium already contains L-Glutamine.
Activity	Karyotyping Medium - BM is tested for sterility, pH, osmolality and endotoxin concentrations.
Use	For the culture of bone marrow cells without any mitogens or conditioned medium.
Storage	Store at $\leq$ -18 ^{o}C. Protect from Light. After thawing, the medium should be stored at 2-8 ^{o}C. The medium should be used within 10 days after thawing. Do not use if a visible precipitate is observed in the medium.
Expiry	Do not use beyond expiration date indicated on the product label.

Use of Karyotyping Medium - BM does not guarantee the successful outcome of any chromosome analysis.

Protocol

Culture of Bone Marrow Cells 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 24-72 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.

1. Inoculate approximately 0.5ml of bone marrow suspension into a plastic tube or tissue culture plate with 5-10ml of Karyotyping Medium – BM (Catalog# IS1012). Invert tubes gently to mix specimen.
2. Incubate the culture at 37 ^{o}C in 5% CO_2 atmosphere for 24-72 hours.
3. Add 0.1-0.2ml of Colcemid Solution 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 5-10 ml of hypotonic 0.075M KCI (Catalog# IS1006). Incubate at 37 ^{o}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 ^{o}C for 10 minutes.
8. Repeat step 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, and the most common method to obtain this staining is to treat slides with Trypsin-EDTA, 10X (Catalog# IS1008).

Optimal dilution and reaction conditions to be determined by investigator usage.