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Karyotyping Medium - HC (with conditioned medium)

Catalog No.	IS1013 100 ml
Introduction	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. Karyotyping Medium - HC 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 polycythernia vera. Karyotyping Medium - HC is based on MEM-Alpha basal medium supplemented with L-Glutamine, fetal bovine serum, antibiotics (penicillin and streptomycin) and conditioned medium.
Format	Karyotyping Medium - HC is supplied as frozen medium, which is ready for use after thawing.
Reconstitution	Thaw Karyotyping Medium - HC at refrigerator temperatures (2-8 ^{o}C) or at room temperature. Mix gently after thawing. Note that the medium already contains L-Glutamine.
Activity	Karyotyping Medium - HC is tested for sterility, pH, osmolality and endotoxin concentrations. In addition, each lot is tested for leukemic cell growth.
Use	For bone marrow and peripheral blood hematopoietic cells
Storage	Store at –20^{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 - HC does not guarantee the successful outcome of any chromosome analysis.

Protocol

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 non-lymphocytic 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.

1. Inoculate approximately 0.5ml of bone marrow suspension or 0.5-1x10^7 Ficoll-separated peripheral blood cells into a plastic tube or tissue culture plate with 10ml of Karyotyping Medium – HC (Catalog# IS1013). Invert tubes gently to mix specimen.
2. Incubate the culture at 37^{o}C in 5% CO_2 atmosphere for 72-120 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 5-10ml 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.