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CHANG Marrow Recommended Protocol

Please refer to the Product Insert for detailed instructions and additional information.

PRODUCT DESCRIPTION

CHANG Marrow is a complete, ready-to-use medium developed for the primary culture of clinical human bone marrow cultures for karyotyping and other genetic testing of various hematological disorders.

STORAGE AND STABILITY

Store frozen below -10°C. Product is stable until the expiration date on the bottle label when stored frozen. Unused product can be dispensed into working aliquots and refrozen for later use, or tightly capped and stored at 2–8°C for up to 30 days; it may be frozen a maximum of two times. Protect from fluorescent light.

PREPARATION FOR USE

Thaw overnight in the refrigerator (2–8°C) then gently mix to ensure homogeneity. Aseptically dispense 10 mL of medium into sterile culture flasks and equilibrate to 37°C for immediate use.

CHANG Marrow contains gentamicin (50 mg/L). Additional antibiotics may be added if desired.

DIRECTIONS FOR USE

Sample Preparation:

Use 0.5–1.0 mL of sodium heparinized bone marrow aspirate. Lithium heparin, EDTA, or citrate anticoagulants are unsuitable for cytogenetic studies.

- If more than 5 mL of bone marrow aspirate is received, the sample may be hemodilute. Spin specimen down to isolate the bone marrow fraction.
- If specimen arrives in transport medium, spin sample down at 1,200 rpm for 8 minutes, then remove the transport medium (supernatant). Inoculate using the remaining spun-down fraction at bottom of tube.

Bone Marrow Culture:

Label all culture vessels with patient name, specimen number, and culture type. For each culture prepare a flask containing:

1. 10.0 mL CHANG Marrow.
2. Equilibrate flask to 37°C before inoculation of specimen.
3. Using a hemocytometer, perform a white blood cell (WBC) count of the sample. Inoculate each culture with they appropriate amount of sample to achieve an optimal concentration of 1 x 10^6 cells/mL or 10 x 10^6 cells per 10 mL culture.
4. Each individual laboratory should determine the number of cultures to set up depending on the clinical indication of the patient. Additional growth factors may be added if desired.
5. Place all of the flasks in a 37°C incubator until ready to harvest.

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Harvesting the Cultures:

1. Remove cultures from incubator and gently swirl to resuspend cells.
2. Transfer the contents of each flask to a 15 mL centrifuge tube.
3. Add 100 µL of stock Colcemid (10 µg/mL) to each tube.
4. Cap tubes and mix by inverting.
5. Incubate tubes at 37°C for 20 minutes.
6. After incubation, centrifuge tubes for 8 minutes at 1,200 rpm (300 x g).
7. Carefully aspirate supernatant from each tube.
8. Resuspend cell pellet by gently mixing, or flicking bottom of tube with forefinger.
9. VERY SLOWLY add 10 mL of hypotonic solution (0.075M Potassium Chloride), to each tube while vortexing (on the lowest setting).
10. Let tubes stand at room temperature for 20 minutes (hypotonic treatment).
11. Centrifuge tubes for 8 minutes at 1,200 rpm (300 xg).
12. Aspirate supernatant leaving about 1.0 mL of hypotonic solution above cell pellet.

Be cautious of fibrous material that may extend from the cell pellet up into the supernatant after centrifugation. The last few mL of supernatant may need to be removed by hand with a Pasteur pipette (not using vacuum aspiration) to avoid aspirating the entire cell pellet into the waste container.

13. Resuspend cell pellet as described in step 8.
14. VERY SLOWLY add 10 mL of 3:1 Methanol:Acetic acid fixative to each tube while vortexing (on the lowest setting).
15. Let tubes stand at room temperature for 20 minutes (first fix).
16. Repeat steps 11–13.
17. Add 5 mL of fixative as in step 14.
18. Let tubes stand at room temperature for 10 minutes (second fix).
19. Repeat steps 16–18 (third fix).
20. At this point, fixed cell pellets can be used immediately for slide preparation according to the laboratory's standard protocol or stored in the refrigerator (2–8°C) for future use.

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