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IrvineScientific

# 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 sulfate (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 hemodiluted. 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.

1. Before inoculation of specimen bring CHANG Marrow to ambient temperature.
2. Inoculate each culture with the appropriate amount of sample to achieve an optimal concentration of 1 x $10^6$ cells/mL or 10 x $10^6$ cells/mL culture.
3. 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.
4. Incubate cultures at 35–39°C, 5–8% $CO_2$ atmosphere until ready for harvest.

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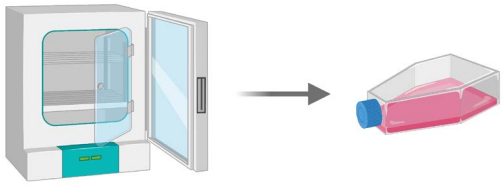
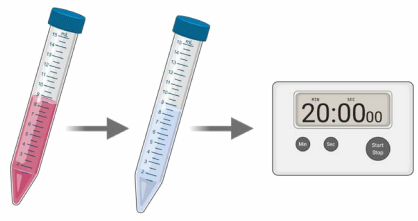
***Harvesting the Cultures:***

1. Remove culture ready for harvest from the incubator and gently swirl flask to resuspend cells.



2. Transfer the contents of each flask to a 15 mL centrifuge tube.
3. Add 40 µL of stock Colcemid (10 µg/mL) to each culture tube. Tightly cap tubes and mix gently by inverting.

4. Incubate tubes at 35–39°C, for 45 minutes.
5. After incubation, centrifuge tubes for 8 minutes at 1,000 rpm.
6. Carefully aspirate supernatant from each tube using a vacuum aspirator, with solvent trap. Be careful to not aspirate pellet.
7. Resuspend cell pellet by tapping bottom or side of each tube with finger.
8. Initiate a 20-minute timer.




9. Add 3–4 mL, dropwise of prewarmed (35–37°C) hypotonic solution (0.075 M Potassium Chloride).
10. Tightly cap tube and mix gently by tapping bottom or side of the tube with finger.
11. Add 5–6 mL, dropwise of prewarmed (35–37°C) hypotonic solution. Tightly cap tube and invert tube.
12. Repeat Steps 9–11 for each tube.
13. Using a water bath, allow tubes to stand at 35–37°C. Invert tubes once at midpoint of 20-minute timer.
14. At the end of the 20-minute timer, remove tubes from water bath and add 1 mL of fresh 3:1 Carnoy's Fixative to each tube. Tightly cap and invert each tube. (This is the Pre-Fixative step.)
15. Centrifuge tubes for 8 minutes at 1,000 rpm.

16. Aspirate supernatant from each tube, leaving about 1 mL above the cell pellet. Be careful to not aspirate 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.

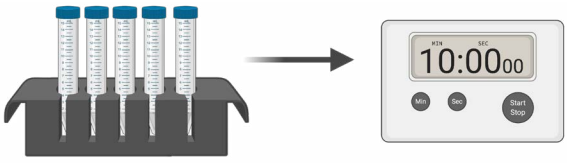
17. Resuspend cell pellet, as described in Step 7.
18. Add 3–4 mL, dropwise of fresh 3:1 Carnoy's Fixative.
19. Add remaining fixative up to 7 mL.

20. Repeat steps 16–19 for each tube.

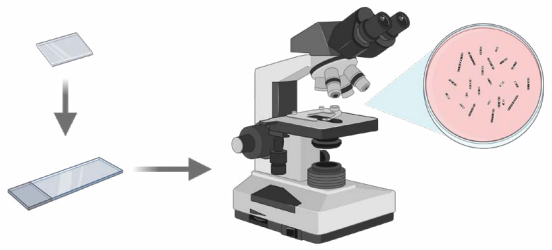
# CHANG Marrow Recommended Protocol

***Continuation:***

21. Let stand for 10 minutes at room temperature. (This is the first fixative step.)




22. Centrifuge tubes for 8 minutes at 1,000 rpm.
23. Aspirate supernatant leaving about 1 mL above pellet. Resuspend cell pellet.
24. Add fix, up to 7 mL. Centrifuge tubes for 8 minutes at 1,000 rpm. (second fixative step.)
25. Repeat steps 22–23. (third fixative step.)
26. At this point, fixed cells pellets can be used immediately for slide preparation according to the laboratory standard protocol or stored in the refrigerator (2–8°C) or freezer for future use.



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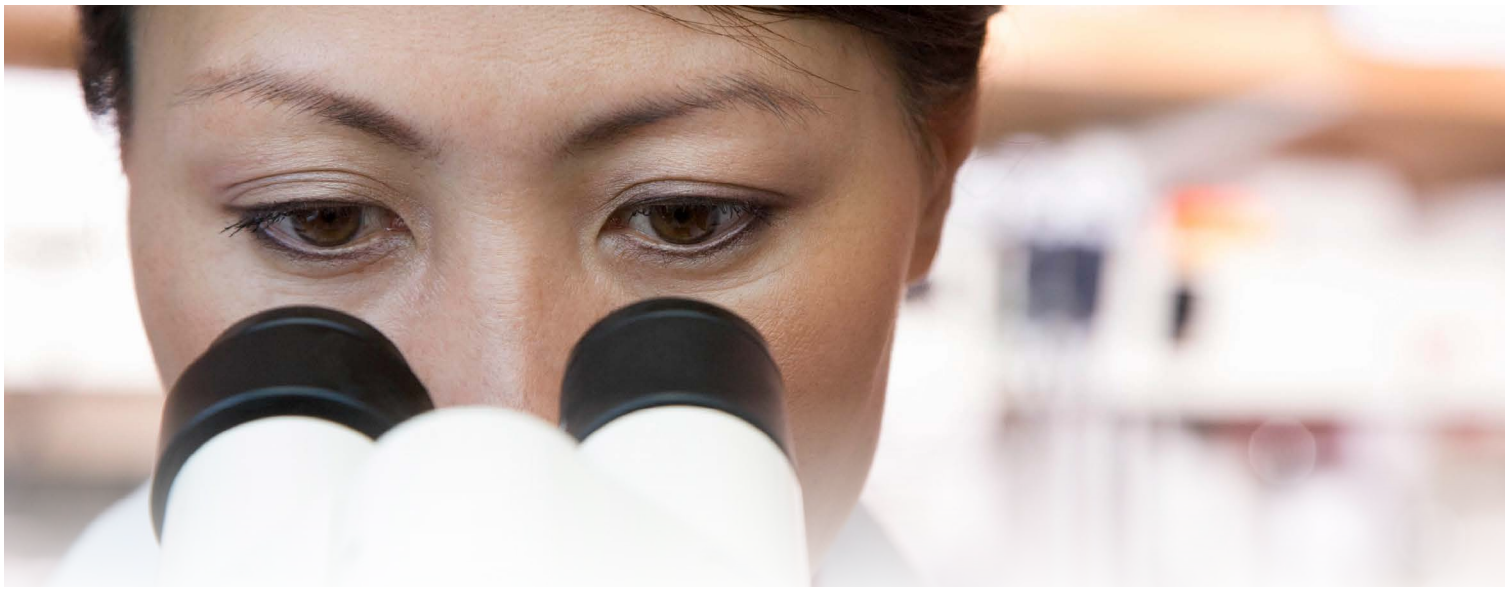

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**FUJIFILM IRVINE SCIENTIFIC – CORPORATE**
1830 E Warner Avenue, Santa Ana, CA 92705 USA
Phone: 1 (949) 261-7800
Toll Free: 1 (800) 437-5706
Fax: 1 (949) 261-6522
Support: fisitmrequest@fujifilm.com