FUJIFILM
Value from Innovation

IrvineScientific

CHANG Medium MF Recommended Protocol

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

PRODUCT DESCRIPTION

CHANG Medium MF is a mitogen-free, ready-to-use medium for use in culturing peripheral blood and other specimens for purposes of cytogenetic analysis.

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

CHANG Medium MF consists of RPMI containing 20% FBS, 2 mM glutamine, 20 mM HEPES buffer and the antibiotic gentamicin sulfate. It may require the addition of mitogenic agents, such as Phytohemagglutinin (PHA) to optimize the growth of peripheral blood and other cells. The required concentration of PHA (or other mitogens) should be determined by the individual laboratory.

DIRECTIONS FOR USE

Sample Preparation:

Obtain 5–10 mL for adults, of fresh blood in sodium heparin, and 2–3 mL for pediatrics.

- Whole blood should be received at room temperature and mixed by inversion.
- Lymphocytes from whole blood can be stimulated with PHA and may be cultured with a synchronization technique.

Whole Blood Culture:

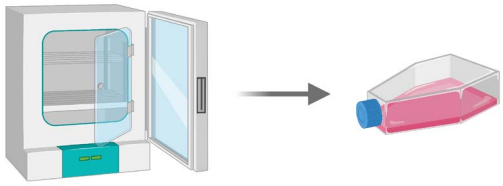
Label all culture vessels with patient name, specimen number, and culture type.

1. Before inoculation of specimen bring CHANG Medium MF to ambient temperature.
2. Reconstitute PHA by adding 5 mL sterile distilled water using a sterile syringe.
3. Prepare required 5 mL of CHANG Medium MF necessary for each culture vessel, using aseptic technique.
4. Add 0.1 mL of reconstituted PHA per culture vessel.
5. Inoculate 0.3 mL of sample per culture. If the patient is an infant (<1 mo old), inoculate with 0.2 mL of sample.
6. Each individual laboratory should determine the number of cultures to set up depending on the clinical indication and age of the patient. An additional 48-hour culture is to be initiated on specimens from newborns (<1 mo old) without synchronization.
7. Incubate cultures at 35–39°C, 5–8% CO_2 atmosphere until ready for harvest.
8. **For synchronization:** After 48 hours of incubation add 50 µL per 5 mL of working solution Methotrexate to each culture to be harvested at 72 hours.
9. Add 100 µL per 5 mL, working solution Thymidine to each culture 18–19 hours after adding Methotrexate (5–6 hours before harvest).

CHANG Medium MF Recommended Protocol

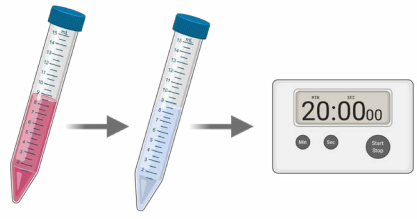
Harvesting the Cultures:

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.

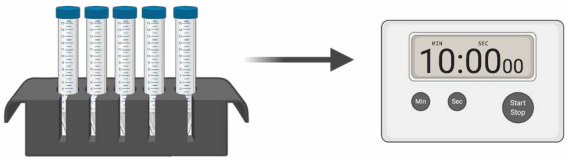
17. Resuspend cell pellet, as described in Step 7.
18. Add 3–4 mL, dropwise of fresh 3:1 Carnoy's Fixative.
19. Add remaining fixative up to 7 mL.

20. Repeat steps 16–19 for each tube.

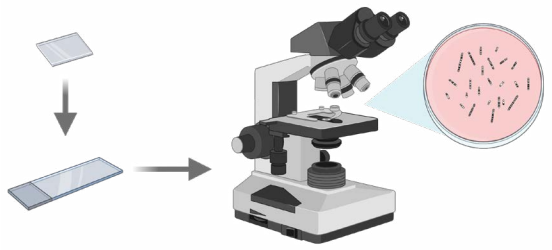
CHANG Medium MF Recommended Protocol

Continuation:

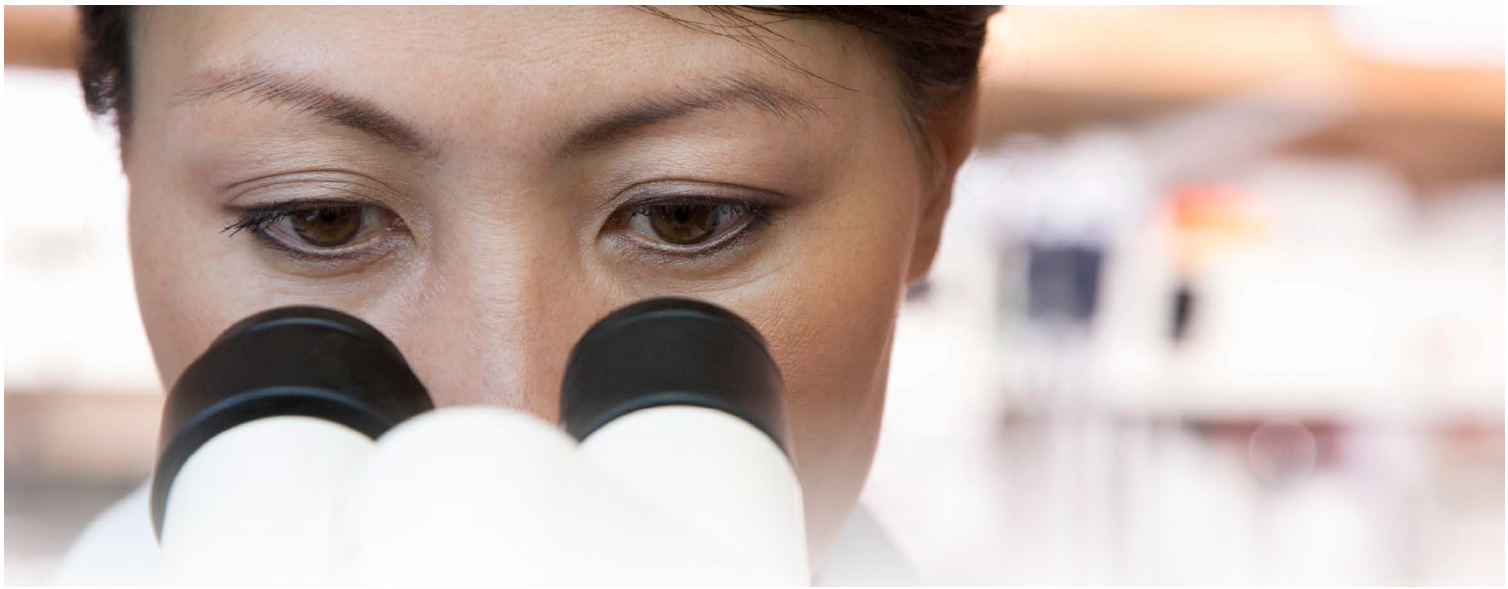
21. Let stand for 10 minutes at room temperature. (This is the first fixative step.)



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.



CHANG Medium MF Recommended Protocol



DISCOVER OUR INDUSTRY EXPERTISE

Partnering with FUJIFILM Irvine Scientific enables your cytogenetics laboratory to leverage 50 years of experience and expertise in cell culture, and delivers the personal attention and expert level of industry knowledge you require. Our team is fully engaged, responsive to your needs, and passionate about your success.

We are by your side for any questions or concerns you may have related to FUJIFILM Irvine Scientific products.

We provide information as needed including:

- Product storage, usage, shelf life and/or stability
- Culture conditions and protocols
- Product specifications, performance, functions, and recommendations
- IFU and documentation requests

Discover our expertise. Learn more about FUJIFILM Irvine Scientific cytogenetics products today—and when you're ready, don't hesitate to contact our cytogenetics experts.
www.irvinesci.com/contact-us

www.irvinesci.com

FUJIFILM
Value from Innovation

IrvineScientific

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