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. 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

I. Preparation of Whole Blood Microcultures

1. Collect 5–20 mL of fresh blood in phenol-free heparin and mix by inversion.
2. Reconstitute PHA by adding 5 mL sterile distilled water using a sterile syringe.
3. Using aseptic technique, prepare required volume of micro-culture medium allowing 5 mL for each blood sample.
4. Dispense microculture medium to appropriately labeled sterile screw-capped tubes and aseptically add 0.1 mL reconstituted PHA.
5. Immediately before culture, add 0.4 mL of heparinized blood using a sterile 1 mL pipette. Use 0.3 mL of blood for infants and children and 0.5 mL for pregnant women.
6. Incubate the cultures at 37°C for 72 hours. For samples from infants and children, incubate one of the two cultures at 37°C for 48 hours. For pregnant women, one of the two cultures can be incubated at 37°C for 96 hours. Mix each tube by inversion daily.
7. Add 0.05 mL (50 µL)/5 mL of working solution of methotrexate to each culture 16–18 hours before addition of thymidine.
8. Add 0.1 mL (100 µL)/5 mL of working solution of thymidine to each culture after completion of methotrexate synchronization (5–6 hours before harvest).

CHANG Medium MF Recommended Protocol

II. Harvesting the Cultures:

1. Pre-warm the hypotonic solution (75 mM potassium chloride) in a 37°C water bath prior to harvest.
2. Add 0.5 µL of a solution of Colcemid to each culture.
3. Mix gently and return to the 37°C incubator.
5. Remove the cultures from the incubator and transfer to plastic graduated centrifuge tubes marked with sample details.
6. Centrifuge the cultures in a bench centrifuge at 500 xg for 5 minutes.
7. Remove most of the supernatant fluid and discard.
8. Resuspend the pellet in 6 to 8 mL of 75 mM potassium chloride solution pre-warmed to 37°C for 10 minutes.
9. Centrifuge as in step 5 and discard supernatant.
10. Using a Pasteur pipette slowly add 6 to 8 mL of freshly prepared modified Carnoy's fixative (3 parts absolute methanol : 1 part glacial acetic acid) to the pellet while agitating constantly on a vortex mixer. Add the fixative drop-wise at first, followed by a slow trickle to minimize cellular damage and formation of lumps.
11. Leave at 4°C for 10 minutes.
12. Centrifuge lightly, remove the supernatant fluid as before and slowly add an additional 5 mL of acetic alcohol to resuspend pellet.
13. Repeat step 12 twice more, resuspending finally in 0.5 mL acetic alcohol. Use this cell suspension to prepare slides for examination. Care must be taken to avoid agitation of the cells.

III. Preparation of Slides:

1. Slides must be scrupulously clean. A suitable cleaning procedure is to soak the slides in chromic acid overnight, after which they should be washed in running water for at least half an hour and polished with a glass cloth.
2. Apply 1 or 2 drops of the resuspended cell preparation to the center of a glass slide from 3–4 inches above top of the slide and allow to spread.
3. Wipe excess fixative from the edges of the slide with filter paper.
4. At the first appearance of Newton's Rings, blow gently to speed final drying of the slide.
5. Stain with Giemsa or 2% acetic acid-orcein stain and mount.

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: tmrequest@irvinesci.com