Product Information, Instructions for Use

MarrowPrime
Complete Medium for Bone Marrow Cells
Cat. No.: MP-B (100 ml), MP-G (20ml)

Product Description

MarrowPrime Medium is intended for *in vitro* use only and has been designed for establishing cultures of bone marrow and leukaemic blood cells, which may then be used in karyotyping, fluorescence in-situ hybridization (FISH) and other cytogenetic procedures. In addition, this product supports highly efficient cell attachment and cell growth which allows fast chromosome analysis.

The medium is supplied frozen as a complete medium, ready to use in a 100 ml format. It is based on MEM Alpha Modification and contains antibiotics (gentamycin), L-glutamine, fetal bovine serum (FBS), hormones and growth factors. It is buffered with sodium bicarbonate and phenol red is present as a pH indicator.

Product Specifications

CO_2 concentration, optimum	5.0 %
pH	7.0 – 7.5
Tumor cells (permanent) cell growth	Positive
Induced Aberrations	Negative
Sterility	Tested
Storage	Store at ≤-15°C. After thawing, the medium should be stored at +2°C to +8°C. The medium should be used within 2 weeks after thawing. Protect the medium from light.

Instructions for use

MarrowPrime is a complete medium, provided in a frozen, sterile format.

Thawing

Thaw MarrowPrime Medium at +37°C in a water bath and mix gently during and after thawing to obtain a homogeneous medium.
An alternative is to thaw medium in a +37°C CO_2 incubator with the lid slightly opened to allow automatic pH normalization. Warm medium at the appropriate pH is best for the initialization of cultures.

Stability

- Store MarrowPrime at ≤-15°C.
- Do not use this product after its expiry date.
- MarrowPrime can be used up to 2 weeks after thawing if stored at +2°C to +8°C.
- Please avoid repeated warming and cooling cycles and exposure to light.

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Protocol for Use

***Important information*:** Supplementation of MarrowPrime Medium is neither necessary nor recommended.

This high quality medium can be used within established procedures. It is up to the user to adopt either parts or all of the optimized protocol described below.

Protocol for setting up and culturing bone marrow cells

1. If a bone marrow sample is received in transport medium, centrifuge at 150 to 170 g for 10 minutes. For bone marrow sample received in heparin, go directly to step 3.
2. Carefully remove the supernatant, including any fat and debris floating on the surface, and discard. Do not affect the pellet.
3. Place 5 ml of MarrowPrime Medium into each tube.
4. Seed with the appropriate amount of bone marrow cells using sterile Pasteur pipettes. The final concentration of cells should be 10^6 cells/ml per culture.
5. Set up cultures according to provisional diagnosis:

a)	*Direct culture:*	Add 100 µl of colcemid solution (10 µg/ml) for 1 to 2 hours.
b)	*Short term culture:*	Incubate overnight. The following morning, add 100 µl of colcemid solution (10 µg/ml) for 1 to 2 hours.
c)	*Overnight exposure to colcemid:*	Add 50 µl (10 µg/ml) of colcemid solution as late in the day as possible. Incubate overnight at +37°C.
d)	*Short term culture + overnight exposure to colcemid:*	Incubate at +37°C for 24, 48 or 72 h. Then add 50 µl (10 µg/ml) of colcemid solution as late in the day as possible. Incubate overnight at +37°C.
e)	*B-cell stimulated cultures :*	Add 100 µl PMA (4-phorbol 12-myristate 13-acetate) and/or PWM (Pokeweed Mitogen) and incubate for 2 to 4 days at +37°C. Add 100 µl of colcemid solution (10 µg/ml) and incubate overnight at +37°C.
f)	*T-cell stimulated cultures :*	Add 100 µl PHA (phytohaemagglutinin) and incubate 72 hours at +37°C. Add 100 µl of colcemid solution (10 µg/ml) for 1 to 2 hours.

Harvesting protocol for bone marrow cells

1. Tubes are centrifuged for 5 minutes at 1500 rpm.
2. Remove supernatant.
3. Resuspend pellet.
4. Add 6 ml of pre-warmed potassium chloride solution (KCl, 0.075 M) and incubate tubes at +37°C in a waterbath for 20 minutes.
5. Centrifuge tubes at 1500 rpm for 5 minutes.
6. Remove supernatant.

7. Add 5 ml of fixative (3 methanol : 1 acetic acid) to the tube. Slowly add a few drops of fixative, mixing gently. Continue adding fixative in this way until all cell clumps have disintegrated and the cell suspension is as homogeneous as possible.
8. Centrifuge at 1500 rpm for 5 minutes.
9. Repeat steps 9-10 two times.
10. After last washing step, carefully remove supernatant without affecting the pellet. Resuspend pellet in appropriate volume of fixative for slide-preparing.

Precautions and Disclaimer

- For *in vitro* diagnostic use. The medium is not intended for therapeutic use.
- Use of MarrowPrime Medium does not guarantee the successful outcome of any diagnostic testing.
- Do not use MarrowPrime Medium beyond the expiration date indicated on the product label.