Product Information

MarrowPrime 2
Complete Medium for Bone Marrow Cells
Cat. No. MP2-B (100 ml)

Intended purpose

The cell culture medium MarrowPrime 2 supports the growth of bone marrow and leukemic blood cells intended for subsequent chromosome analysis (karyotyping, fluorescence *in situ* hybridization and other cytogenetic procedures).

General Information

MarroPrime 2 is a newly designed and optimized formulation. With years of know-how, we have developed a formulation which results in a better performance and longer stability.

MarrowPrime 2 Medium supports highly efficient cell attachment and cell growth which allows fast chromosome analysis of bone marrow and leukemic blood cells and is intended for *in vitro* use only. MarrowPrime 2 is a non-automatic, *in vitro* Diagnostic class A sterile product intended for laboratory use.

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 (gentamicin), 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.

Application:

- For karyotyping, fluorescence in-situ hybridization and other cytogenetic procedures of established bone marrow and leukemic blood cell cultures

Product Specifications

Appearance	Clear yellow to red frozen liquid
Composition	Basal medium, pretested FBS, hormones and growth factors, phenol red, buffering by $NaHCO_3$; Gentamycin and L-glutamine.
Storage and shelf life	Store at ≤-15°C protected from light. Do not use this product after its expiry date. Once opened, store at +2°C to +8°C and use within 2 weeks.
Shipping conditions	Frozen (dry ice)
Thawing	Thaw MarrowPrime 2 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.**

For lot specific data (Certificate of Analysis) please refer to our website:
https://www.capricornscientific.com/en/services/certificate-of-analysis

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Instruction for Use

Important information:

MarrowPrime 2 is a complete medium, provided in a frozen, sterile format.
Supplementation of MarrowPrime 2 Medium is neither necessary nor recommended.

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

Setting up and culturing of 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 2 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:

Direct culture:	Add 100 µl of colcemid solution (10 µg/ml) for 1 to 2 hours.
Short term culture:	Incubate overnight. The following morning, add 100 µl of colcemid solution (10 µg/ml) for 1 to 2 hours.
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.
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.
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.
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 of bone marrow cells:

1. Tubes are centrifuged for 5 minutes at 1500 rpm.
2. Remove supernatant.
3. Resuspend pellet in 6 ml of pre-warmed potassium chloride solution (KCl, 0.075 M) and incubate tubes at +37°C in a water bath for 20 minutes.
4. Centrifuge tubes at 1500 rpm for 5 minutes.
5. Remove supernatant.
6. 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.
7. Centrifuge at 1500 rpm for 5 minutes.
8. Repeat steps 6-7 two times.
9. After last washing step, carefully remove supernatant without affecting the pellet. Resuspend pellet in appropriate volume of fixative for slide-preparing.

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Precautions and Disclaimer

- For *in vitro* diagnostic use. The medium is not intended for therapeutic use with humans or animals.
- Application only by trained specialist personnel.
- Maintaining the sterility of the product is necessary for its use and must be strictly observed by the user.
- Do not use MarrowPrime 2 medium if the packaging is damaged and thus sterility is impaired.
- Each laboratory is obliged to perform representative tests according to the valid legal regulations and in its own environment to ensure that it is suitable for this purpose before the medium can be used in routine diagnostics.
- The patient specimens are biological material and therefore safety precautions must be taken according to local regulations for working with potentially infectious material.
- Use of MarrowPrime 2 medium does not guarantee the successful outcome of any diagnostic testing.
- Do not use MarrowPrime 2 medium beyond the expiration date indicated on the product label.
- Report serious incidents that have occurred in connection with this product to the manufacturer and the appropriate authorities.

Important Note

Occasionally, the formation of calcium oxalate crystals is possible, but these have not shown any negative influence on cell growth.

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).