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ProductInformation

BONE MARROW MEDIUM PLUS

Product Code **B 6301**
Storage Temperature –20 °C

Product Description
Sigma's Bone Marrow Medium Plus (Product Code B 6301) has been developed for *in vitro* short-term growth of bone marrow cells for chromosome analysis. This formulation consists of an RPMI-derived basal medium supplemented with fetal bovine serum, and antibiotic. The Bone Marrow Medium Plus also contains Giant Cell Tumor (GCT) conditioned medium. This product is intended for the culture of cells from bone marrow aspirates. This medium may also be used to culture leukemic blood specimens, when circulating blasts are present.

Components
Basal Medium: RPMI 1640 with L-glutamine
Buffers: HEPES and Sodium Bicarbonate
Serum: Fetal Bovine Serum, optimized
Growth Factors: GCT conditioned medium
Antibiotic: Gentamicin Sulfate

Intended Use
For *In Vitro* diagnostic use.

Storage/Stability
Sigma's Bone Marrow Medium Plus should be stored in the dark at freezer temperatures (-20 °C). After thawing, medium should be kept at refrigerated temperatures (2-8 °C). DISCARD THE MEDIUM WITHIN 10 DAYS AFTER THAWING. Frost-free freezers and repeated freeze thaw cycles can accelerate product breakdown and should be avoided. Avoid exposure to light. Any or all of the following may be recognized as deterioration of the medium: [1] color change, [2] cloudiness, [3] pH change and [4] diminished cell growth and poor chromosome morphology. Label bears expiration date.

Procedure
1. Thaw medium overnight at refrigerated temperatures (2-8 °C). Mix gently.
2. Dispense medium into smaller aliquots and store at freezer temperature (–20 °C) for later use, OR warm medium to 37 °C and use immediately for bone marrow cultures following standard laboratory procedures.
3. A recommended protocol for the culture and harvest of bone marrow cells is given above. Detailed protocols for all of Sigma's cytogenetics products are also available at Sigma-Aldrich's Web site: **sigma-aldrich.com**.

Recommended protocol for the culture and harvest of bone marrow specimens:
1. Inoculate approximately 500 µl of bone marrow suspension or the appropriate amount of bone marrow based on the patients white cell count into tubes containing 10 ml of Bone Marrow Media Plus (Product Code B 6301). Cultures may be supplemented with growth factors and/or mitogens according to your own laboratory standards for bone marrow culture.
2. Invert tubes to thoroughly mix specimen.
3. Incubate cultures at 37 °C and 5% CO_2 for 24 to 48 hours.

Harvest of bone marrow cultures:
1. Remove cultures from incubator and invert several times to resuspend the cells, as they may have settled during culture.
2. To each culture tube add 100 µl of Demecolcine (10 µg/ml, Product Code D1925).
3. Invert tubes to mix solution and incubate at 37 °C for 20 minutes.
4. After incubation, spin tubes at 1,000 rpm for 10 minutes.
5. Aspirate the supernatant from each tube leaving approximately 0.5 ml above each pellet.
6. Resuspend the pellets by gently mixing.
7. Add 10 mls of pre-warmed (37 °C) hypotonic solution (0.075 M Potassium Chloride, Product Code P 9327) to each culture, then incubate tubes for 20 minutes at 37 °C.
8. Following incubation, add 1 ml of Carnoy's fixative [75% methanol (Product Code M3641) : 25% Glacial Acetic Acid, (Product Code A 6283)] to each culture and invert to distribute evenly.
9. Let the cultures sit for 5 minutes at room temperature.
10. Spin the cultures at 1000 rpm for 10 minutes.

11. Aspirate all but 1 ml of the supernatant from each tube.
12. Gently mix pellets with remaining supernatant before adding 10 ml of Carnoy's fixative to each suspension.
13. Let the cultures stand in the fixative for at least 30 minutes. If necessary the cultures may sit overnight before moving to the next step.
14. Centrifuge the tubes at 1,000 rpm for 10 minutes.
15. Aspirate all but 1 ml of the supernatant from each tube.
16. Add 5 ml of fresh Carnoy's fixative to each culture.
17. Repeat steps 14-16.
18. Fixed cell pellets can then be used immediately to prepare chromosome spreads according to your own laboratory standard protocol. Pellets may also be stored for future use.

Product Profile

Appearance	Clear red solution
pH	7.2 ± 0.2
Osmolality	290 mOsm/kg H_2O ± 5%
Sterility bu USP	Sterile
Cell Culture Test	Pass

References

1. Barch MJ., Knutson T., Spurbeck, J.L., Eds. The ACT Cytogenetics Laboratory Manual, 3rd edition. New York: Raven Press, 1997
2. Ronney D.E., Czepulkowski B.H. Human Cytogenetics, A Practical Approach , 2nd Ed. Volume 1, Constitutional Analysis, Oxford: IRL Press, 1992
3. Castagne C., Muhlematter D., van Melle G., Gachoud V., and Jotterand Bellomo M. Effect of Conditioned Media, Nutritive Elements, and Mitotic Synchronization on the Accuracy of the Cytogenetic Analysis in Acute Non-lymphocytic Leukemia Patients Presenting with inv (16)/t(16;16) or t(15;17). Cancer Genetics and Cytogenetics 1997; **94**:106-112.

Precautions and Disclaimer

REAGENT

For *In Vitro* Diagnostic Use

1. Do not use if product is received thawed or shows visible precipitate.
2. Do not dilute or mix product with other media for it will interfere with product performance.
3. Product is not intended for therapeutic use.
4. Use of Sigma's Bone Marrow Medium Plus does not guarantee successful diagnostic procedures.

MSDS is available upon request at: **sigma-aldrich.com**.

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