

Product Information

BONE MARROW TRANSFER SOLUTION

Product Number **B6426**
Store at Room Temperature

Product Description

Cytogenetic analysis of bone marrow aspirates is an integral part of cancer diagnostics. However, the extreme variability of sample size and quality often complicates the diagnostic process. Sigma's Bone Marrow Transfer Solution has been developed to facilitate the recovery of bone marrow aspirates by providing a liquid matrix for the short-term preservation and transportation of specimens. The volume of transfer solution employed provides a more uniform concentration of heparin, independent of sample size, which protects the specimens from clotting or potential toxic effects. This product is ideal for low volume specimens, as it will also provide protection from specimens drying on the walls of collection tubes. The product is supplied in single use tubes for easier specimen collection and processing.

Components

Basal Solution	Balanced Nutrient Solution
Buffers	HEPES and Sodium Bicarbonate
Anti-Coagulant	Sodium Heparin

Precautions and Disclaimer

REAGENT

For In Vitro Diagnostic Use

1. Product should not be used to culture bone marrow cells.
2. Do not use if product shows visible precipitate, is cloudy or has a yellow color to it.
3. Do not dilute product with other media or add supplementation before use. It may interfere with product performance.
4. Product is not intended for therapeutic use.
5. Use of Sigma's Bone Marrow Transfer Solution does not guarantee successful diagnostic procedures.

Storage/Stability

Sigma's Bone Marrow Transfer Solution should be stored at room temperature. Label bears expiration date.

Procedure

Specimen Collection and Transportation:

1. Store tubes containing Sigma's Bone Marrow Transfer Solution at room temperature.
2. Label tubes before use.
3. Remove cap and aseptically transfer bone marrow aspirate into tube. Up to 2 ml of aspirate can be added to each tube. Tighten cap onto tubes.
4. Mix tubes gently to ensure aspirate is diluted into transfer solution and washed off the wall of the tube.
5. Protect specimens from extremes of temperature during transport and shipping.
6. Store specimens at room temperature upon receipt. **Do not refrigerate or freeze specimens.**
7. Do not store specimens in transfer solution for more than five days after collection.
8. Detailed recommended protocol for culture and harvest of bone marrow cells is listed below. Protocols for all of Sigma's Cytogenetics Products are available on Sigma's web page: [www.sigma-aldrich.com].

Recommended protocol for the culture of bone marrow specimens transported in Sigma's Transfer Solution:

1. Centrifuge the tubes containing both specimen and transfer solution at 1000 rpm for 10 minutes.
2. Carefully aspirate the transfer solution supernatant off the bone marrow without disturbing the pellets.
3. Resuspend each pellet in a small amount, approximately 250 μ l, of culture medium.
4. Inoculate approximately 500 μ l of bone marrow suspension or the appropriate amount of bone marrow based on the patient's white cell count into tubes containing 10 ml of culture medium, such as Sigma's Bone Marrow Medium (Product No. B6176) or Bone Marrow Medium Plus (Product No. B6301). Cultures may be supplemented with growth factors and/or mitogens according to your own laboratory standards for bone marrow cultures.

5. Invert tubes to thoroughly mix specimen.
6. Incubate cultures at 37 °C and 5% CO₂ 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 μ l of Demecolcine (10 μ g/ml, Product No. 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 ml of pre-warmed (37°C) hypotonic solution (0.075 M Potassium Chloride, Product No. P9327) 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 No. M3641) : 25% Glacial Acetic Acid, (Product No. A6283)] to each culture and invert tubes 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 pellet 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 tube.
17. Repeat steps 14-16.
18. Fixed cell pellets can then be used to prepare chromosome spreads according to your own laboratory standards.

Product Profile

Appearance	Clear red solution
pH at room temperature	7.2 ∇ 0.3
Osmolality	290 mOsm/kg H ₂ O ∇ 5%
Sterility by USP XXIII	Sterile
Cell culture assay	Non-toxic

References

1. Barch MJ., Knutson T., Spurbeck, J.L., Eds. The ACT Cytogenetics Laboratory Manual, 3rd edition. 1997, New York: Raven Press
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