ProductInformation

PERIPHERAL BLOOD MEDIUM
Product Code **P 2602**
Storage Temperature –20 °C

Product Description
Peripheral Blood Medium (Product Code P 2602) has been developed for in vitro short-term growth of peripheral blood lymphocytes for chromosome analysis. The formulation consists of an RPMI-derived basal medium supplemented with fetal bovine serum, L-glutamine, phytohemagglutinin and antibiotic. It is supplied as a ready-to-use frozen product. This medium is complete and needs no additional supplementation.

Components
Basal: RPMI-1640 with L-glutamine

Buffers: HEPES and Sodium Bicarbonate

Serum: Fetal Bovine Serum, optimized

Mitogens: Phytohemagglutinin (PHA), optimized

Antibiotic: Gentamicin Sulfate

Intended Use
For In Vitro Diagnostic use
Product is not intended for therapeutic use.

Storage/Stability
Peripheral Blood Medium should be stored in the dark at freezer temperatures (-20 °C). Do not use if product is received thawed or shows a visible precipitate. After thawing, medium should be kept at refrigerator 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 refrigerator temperatures (2-8 °C). Mix gently.
2. Dispense medium into smaller aliquots and store at freezer temperature (-20 °C) for later use.
3. To use immediately after thawing, warm medium to 37 °C and use for blood culture following standard laboratory procedures.

Recommended protocol for the culture and harvest of peripheral blood specimens:
1. Inoculate 500 µl of heparinized whole blood into tubes containing 10 ml of Sigma's Peripheral Blood Medium (Product Code P 2602).
2. Invert tubes to thoroughly mix specimen.
3. Incubate cultures at 37 °C and 5% CO_2 for 72 hours.
4. Recommended protocols for all of Sigma's Cytogenetic Products are also available on Sigma-Aldrich's Web site: **sigma-aldrich.com.**

Harvest of peripheral blood 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 D 1925).
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 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 M 3641):25% Glacial Acetic Acid, (Product Code A 6283)] 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 1,000 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 by USP	Sterile
Cell Culture Assay	Pass

References

1. Barch MJ., Knutson T., Spurbeck, J.L., Eds. The ACT Cytogenetics Laboratory Manual, 3rd edition. New York: Raven Press, 1997
2. Rooney D.E., Czepulkowski B.H., Eds. Human Cytogenetics - A Practical Approach, 2nd Ed. Volume 1 - Constitutional Analysis, Oxford: IRL Press, 1992
3. Donovan M.R.O., Johns S., Wilcox P. The effect of PHA stimulation on lymphocyte sub-populations in whole blood cultures. Mutagenesis 1995; 10: 371-374.

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

Precautions and Disclaimer
Do not dilute or mix product with other medium, alteration can interfere with product performance. Use of this Peripheral Blood Medium does not guarantee successful diagnostic procedures.

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