MarrowGrow Medium

CE

Product Information

MarrowGrow Medium	MGM-100	100 ml

CAUTION

Handle in accordance with established bio-safety practices.

MarrowGrow Medium

Product Information

Cat. No. MGM - 100 (100ml frozen)

Product Description

MarrowGrow Medium is intended for *in vitro* use and has been designed for establishing cultures of bone marrow and *leukaemic blood* cells, which may then be used in karyotyping, fluorescence *in-situ* hybridisation (FISH) and other cytogenetic procedures.

MarrowGrow Medium can be used as a non-specific medium to culture the different haematopoietic cells (myeloid and lymphoid lineages) present in bone marrow or leukaemic blood sample. MarrowGrow Medium can also be used together with a mitogen specific to B or T lymphocytes where these particular lines are being investigated (see Protocol paragraph for further details).

MarrowGrow Medium is supplied frozen as a complete medium, ready to use in a 100ml format. It is based on alpha-MEM and contains antibiotics, L-glutamine, Foetal Bovine Serum (FBS), a hormone and growth factors. MarrowGrow is buffered with Sodium Bicarbonate and Phenol Red is present as a pH indicator. The complete formulation reduces the chance of technical error and culture contamination. In addition, this product supports more efficient cell attachment and cell growth resulting in early chromosome analysis.

Storage Conditions

Frozen: Store at -20°C and in the dark until the stated expiry date shown on the label.

Refrigerated: Store at +4°C for up to 2 weeks.

Instructions for use

MarrowGrow is a complete medium, provided in a frozen, sterile format. Re-filtering the medium is not necessary. Successful cell cultures depend on the use of good aseptic technique. The following suggestions may be useful in using MarrowGrow.

Thawing

MarrowGrow should be thawed in a 37°C waterbath and mixed by swirling prior to use. An alternative is to thaw medium in a 37°C CO_2 incubator with the lid slightly opened to allow for automatic pH normalisation. Warm medium at the proper pH is best for the initialisation of cultures.

Control of pH

MarrowGrow is supplied at the correct pH 6.9-7.1, but occasionally the pH may fluctuate:

MarrowGrow contains Phenol Red as a pH indicator :

- Pink indicates a high pH. Phenol Red indicator turns pink at pH 7.4. Pink medium (too alkaline) can be corrected by opening the cap a small amount (about ¼ turn) and incubating the bottle in a 5% CO_2 incubator for about 1 hour. The medium will remain sterile and will titrate automatically to the correct pH endpoint.
- Yellow indicates a low pH. Phenol Red indicator turns yellow at pH 6.7. Yellow medium (too acidic) can be corrected the same way as pink media.

- Alternatively, the medium bottle or culture flask can be gassed directly with sterile, 5% CO_2 in air in a sterile hood until the correct colour is obtained.
- The colour can be compared to a fresh bottle of thawed MarrowGrow if necessary.

Antibiotics

MarrowGrow contains Gentamycin, which is less inhibitive to growth compared to Penicillin and Streptomycin.

Stability

- MarrowGrow remains stable for up to the expiry date stated on the label when frozen.
- Do not use beyond the labelled expiry date.
- MarrowGrow can be used up to 2 weeks when thawed and stored at 4°C, but eventually the glutamine will degrade. It can be supplemented once only with L-glutamine up to 2.0mM (1/100 vol. of 200mM glutamine stock).
- Repeated warming/cooling and exposure to light should be avoided.

Performance Testing

MarrowGrow is tested for pH, osmolarity, bacterial, mycoplasm and fungal contamination checks. In addition to these standard specifications, each manufactured lot is tested for cell growth by a leading independent European Cytogenetics laboratory and the product performance is compared to a reference standard. A Certificate of Analysis is available upon request.

Precautions

Please contact directly the Sales & Marketing Department at CytoGen GmbH for any concerns relating to the product.

Do not use product if :

- Packaging appears compromised.
- Product appears cloudy or a visible precipitate is observed.

If product is received thawed or partially thawed, freeze immediately at -18°C and contact CytoGen.

Limitations

Each laboratory must carry out their own testing procedures on new media prior to releasing them to the lab for routine *in vitro* applications. CytoGen's contribution to these procedures is simply to provide a culture or handling medium and therefore CytoGen do not guarantee the successful outcome of any testing based only on the use of CytoGen medium.

Cell Culture Protocols

Choice of method (from Rooney D. E. *et al*).

Several methods are available and the choice of method is highly dependent on the condition being investigated and suggested regimes are given in the table below. These are guidelines.

Diagnosis	**Sample**	**Minimum Cultures**	**Extras**
CGL/CML	PB/BM	ONC, S	D, 48h
AML (except APL)	BM/PB	ONC, S	24h, 48h
APL	BM/PB	ONC, 24h, S	48h
MDS	BM	ONC, S	24h, 72h ONC
MPD	BM	ONC, S	24h, 72h ONC
ALL (non B/T)	BM/PB	ONC, S	D, 24h, 48h
ALL (T cell)	BM/PB	ONC, S	D, 24h, 48h, 3d

ALL (B cell)	BM/PB	ONC, 3d + PMA	D, S, 24h, 48h
		5d + PMA; 2d + PHA	
CLL (B cell)	PB/BM	ONC, 3d + PMA	3d
		5d + PMA; 2d + PHA	
CLL (T cell)	PB/BM	ONC, 3d + PHA	3d
		3d PMA	
T-cell lymphoma	LN/BM	ONC, 3d + PHA	3d
		3d + PMA	
B-cell lymphoma	LN/BM	ONC, 3d + PMA	3d
		5d + PMA	
Other lymphoid disorders	BM	ONC, 3d +/- PMA	S
		5d +/- PMA	

APL : acute promyelocytic leukaemia; MPD : myeloproliferative disorder; PB : peripheral blood; BM : bone marrow; LN : lymph node; ONC : overnight cocemid exposure; S : synchronised culture; PHA : phytohaemagglutinin; PMA : 4-phorbol 12-myristate 13-acetate.

The protocol below provides a guide for bone marrow culture using MarrowGrow. It can be used to replace either part of or all of existing optimised protocols for bone marrow cultures at the user's discretion. The majority of cytogenetic laboratories have their own protocols and MarrowGrow can, in most cases, be simply substituted in current cell culture protocol. **The most common culturing method uses an "open" system.**

Very important : Please note

Definition of "open" system : cultures growing in dishes with vented lids or in flasks/tubes with loosened caps in a 5% CO_2 atmosphere (gas incubator) which allows gaseous exchange.

Definition of "closed" system : cultures growing in a standard ungassed, dry incubator in tightly sealed culture vessels.

Recommendations for use of "open" system

Reagents

- MarrowGrow complete medium
- Colcemid solution (10µg/ml)
- Sodium hypochlorite solution (2.5%)

*Protocol for setting up and culturing bone marrow cells**

1. If a bone marrow sample is received in transport medium or from a patient on a chemotherapy regime, centrifuge at 150-170g for 10 minutes. For bone marrow received in heparin, go direct to step 3.
2. Remove the supernatant, including any fat and debris floating on the surface, and discard. Care must be taken not to disturb the pellet. **
3. Add 5ml of MarrowGrow Plus to each of the tubes in a rack.
4. Seed with the appropriate amount of bone marrow using sterile Pasteur pipettes (according to cell count). The final concentration of cells should be 10^6/ml per culture.
5. Set up cultures according to provisional diagnosis :
    a) Direct cultures : add 100µl of colcemid solution for 1-2 hours
    b) Short term cultures : incubate overnight. The following morning, add 100µl of colcemid solution for 1-2 hours
    c) Overnight exposure to colcemid : add 50µl 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 72h. Then as step 5. c).
    e) B-cell stimulated cultures : add 100µl PMA and/or PWM and incubate for 2-4 days at 37°C. add 100µl of colcemid solution and incubate overnight at 37°C.
    f) T-cell stimulated cultures : add 100µl PHA and incubate 72h at 37°C. add 100µl of colcemid solution for 1-2h.

* Carry out all steps in a Class 2 microbiological safety cabinet, use sterile pipettes unless otherwise specified and discard unfixed material into sodium hypochlorite. Use the appropriate size of pipette for the required volume.

** When a sample has a high white cell count the pellet is very easily disturbed, so great care is required at this stage.

Harvesting protocol for bone marrow cells

1. Tubes are then centrifuged for 5 minutes at 1500rev/min.
2. Remove supernatant.
3. Resuspend pellet.
4. Add 6ml of pre-warmed KCL and incubate tubes at 37°C in waterbath for 20minutes.
5. Centrifuge tubes at 1500rev/min for 5 minutes.
6. Remove supernatant.
7. Add 5ml of fixative (3 methanol : 1 acid acetic) to 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 even as possible.
8. Centrifuge at 1500rev/min for 5 minutes.
9. Repeat steps 9-10 2 times.
10. After the last wash, remove supernatant as close to the pellet as possible without disturbing it, and then resuspend in as much fixative as is required for slide-making.

Recommendation for use of closed system

MarrowGrow can be used to culture cells in a "closed" system as long as the pH remains physiologic (pH = 6.9 to 7.4). Closed systems rely on the intrinsic buffering capacity of the medium in the absence of the benefit provided by the equilibrium between the bicarbonate in the medium and the 5% CO_2 present in an open system incubator. Closed systems work best in cloning applications with low cell density since higher cell densities produce acidic metabolites that can acidify the medium beyond its physiologic buffering capacity. Maintenance of pH can be accomplished in the closed system by one of the 3 following methods :

- Method 1 : Supplement MarrowGrow with 2% (v/v) of sterile 1.0M HEPES stock solution. The sterile 1.0M HEPES must be adjusted to pH 7.0 at 20°C with 1.0M NaOH. The HEPES supplemented medium is then combined with cells and incubated at 37°C with the culture flask closed.
- Method 2 : Pre-equilibrate culture flasks containing MarrowGrow and cells in an open 5% CO_2 incubator for one hour prior to closing the cap and culturing at 37°C.
- Method 3 : Purge each individual culture flask containing MarrowGrow and cells with 5% CO_2 - 95% air from a sterile pipette for 20 seconds. Then close the cap and culture in a closed system at 37°C. (It is recommended that a sterile plugged Pasteur pipette be attached to the CO_2 source to ensure sterility of the incoming gas).

Bibliography: Rooney D. E. and Czepulkowshi B.H. Human Chromosome Preparation. (1997).

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