MARROWGROW™

Complete Karyotyping Medium for the culture of bone marrow and other hematopoietic cells for cytogenetic studies (karyotyping, fluorescence in situ hybridization) and in vitro diagnostics.

Filtration, Treatment	Sterile Filtered; contains preselected FBS, hormones and growth factors, Gentamycin and L-glutamine.
Product Code	MMG-001-100ML
Shelf Life	24 months from DOM
Storage Temperature	Store between-5°C to -20°C protected from light. Once opened, store at +2°C to +8°C and use within 2 weeks
Shipping Temperature	Frozen (Dry ice)
Thawing	+37°C in water bath and swirl gently to homogenize. An alternative is to thaw medium in a +37°C CO2 incubator with the lid slightly opened to allow automatic pH normalization. Warm medium at the appropriate pH is best for the initialization of cultures.

QC Specifications

Physical and Chemical Analysis	**Method**	**Specifications**	**Units**
Appearance	Visual	Clear amber to red frozen liquid	n/a
pH at RT	Electronic pH Meter	6.8 - 7.6	n/a
Osmolality	Osmometer	Test and report	mOsm/kg
Endotoxin	LAL Kinetic	≤ 10.0	EU/ml
Sterility			
Aerobic Bacteria	*EP 2.6.1*	Not detected	n/a
Anaerobic Bacteria	*EP 2.6.1*	Not detected	n/a
Fungi (Yeast & Mold)	*EP 2.6.1*	Not detected	n/a
Mycoplasma	qPCR	Not detected	n/a

GENERAL INFORMATION/FORMULATION

This medium is ready to use product. It has been specifically developed for the cultivation of human bone marrow and other hematopoietic cells, which are intended for the preparation of karyograms, fluorescence *in situ* hybridization and other cytogenetic methods. The medium is supplied frozen.

The medium is formulated based on the basal medium, supplemented with preselected Foetal Bovine Serum, hormones and growth factors, phenol red, buffering by NaHCO3; Gentamycin and L-glutamine. L-Glutamine gentamicin.

INSTRUCTION FOR USE

This medium is ready to use and Supplementation of *Marrowgrow* is neither necessary nor recommended.

Culture of bone marrow cells:

1. If a bone marrow sample is received in a transport medium, centrifuge at 150 to 170 g for 10 minutes. For bone marrow samples 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 touch the pellet.
3. Place 5 ml of *Marrowgrow* 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 106 cells/ml per culture.
5. Set up cultures according to provisional diagnosis:

Culture type	Treatment
Direct culture	Add 100 µl of colcemid solution (10 µg/ml, Serana Product code: *CDS-002-10ML*) 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 of colcemid solution (10 µg/ml, Serana Product code: *CDS-002-10ML*) 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 (Serana Product code: *CDS-002-10ML*) 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-M(Serana Product code: *CDS-001-10ML*) and incubate 72 hours at +37°C. Add 100 µl of colcemid solution (10 µg/ml, Serana Product code: *CDS-002-10ML*) for 1 to 2 hours.

Harvesting of bone marrow cells: Cells:

1. Centrifuge the tubes 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, Serana Product code: *CDS-003-100ML*) 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.

PRECAUTIONS AND DISCLAIMER

- The medium is not intended for therapeutic use.
- Each laboratory must perform representative tests according to 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; therefore, safety precautions must be taken according to local regulations for working with potentially infectious material.
- Do not use if a visible precipitate is observed in the medium.
- Use of the *Marrowgrow* medium does not guarantee the successful outcome of any diagnostic testing.
  Do not use this medium beyond the expiration date indicated on the product label.
- Occasionally, the formation of calcium oxalate crystals is possible, but these have not shown any negative influence on cell growth.

SAFE DISPOSAL

Waste treatment methods

The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

Environmental precautions

Do not let the product enter drains.