Intended use
CytogenMarrow is used for the cultivation of bone marrow and leukaemic blood cells. It is intended for human genetic in vitro diagnostics.

Composition
Basal medium, pre-tested FBS, hormones and growth factors, phenol red, buffering by means of $NaHCO_3$; contains gentamycin and L-glutamine.

Shelf life and storage
Unopened CytogenMarrow medium can be stored for 18 months from the date of manufacture if stored at ≤-18°C. After opening, store the bottle at +2°C to +8°C and use for a maximum of 7 days. Avoid repeated thawing and freezing.

Thawing
Thaw CytogenMarrow Medium at +2°C to +8°C overnight. Thawing in a water bath at 37°C is not recommended. Mix Marrogro Medium well before use. The normal pH is 7.2, as indicated by the phenol red indicator. In the event of a pH deviation (yellow or pink), the pH value is equilibrated by incubation of the bottle, slightly opened (approx. ¼ rotation of the lid) in a 5% CO_2 incubator until the medium has reached the normal colour red. CytogenMarrow Medium does not contain any components whose quality is impaired by pH fluctuations of +/- 2. Warmed medium at 37°C and the correct pH value ensures an optimal start for the culture.

Standard protocol
The methods described below are proven instructions for the use of CytogenMarrow for the cultivation of bone marrow cells. CytogenMarrow Medium is filled under aseptic conditions. Maintaining the sterility of the product is necessary for its use in *in vitro* diagnostics, and this must be strictly adhered to by the user. Of course, this high-quality medium can be integrated into your own workflow. It is up to the user to decide whether the protocols, etc., are transferred completely or only partially into their own protocols.

1. If a bone marrow sample is received in transport medium or from a patient on chemotherapy, centrifuge at 150-170 g for 10 minutes. For bone marrow received in heparin, go directly to step 3.
2. Remove the excess without touching the pellet.
3. Add 5 ml of CytogenMarrow to each of the tubes.
4. Seed with the appropriate amount of bone marrow using sterile Pasteur pipettes (according to the cellcount). The final concentration of cells should be 106/ml per culture.
5. Prepare cultures according to the preliminary diagnosis:
    a) Direct cultures: Adding 100µl Colcemid solution for 1-2 hours.
    b) Short-term cultures: incubate overnight. Add next morning 100µl Colcemid solution for 1-2 hours.
    c) Exposure to colcemid overnight: add 50µl colcemid solution as late in the day as possible.Incubate overnight at 37°C.
    d) Short-term culture + colcemid exposure overnight: incubate for 24, 48 or 72 h at 37 °C. Then as step 5. c).
    e) B-cell stimulated cultures: Add 100µl PMA and/or PWM and incubate at 37°C for 2-4 days. Add100µl colcemid solution and incubate overnight at 37°C.
    f) T-cell stimulated cultures: Add 100µl PHA and incubate for 72 h at 37°C. Add 100µl colcemid solution for 1-2h.

Harvest protocol for bone marrow cells

1. Centrifuge the tubes for 5 minutes at 1500 rpm.
2. Remove the overhang.
3. Resuspend the pellet.
4. Add 6 ml of pre-warmed KCL and incubate the tubes for 20 minutes at 37 °C in a water bath.
5. Centrifuge the tubes for 5 minutes at 1500 rpm.
6. Remove the supernatant.
7. Add 5 ml fixative (3 methanol: 1 acetic acid) to the tube. Slowly add a few drops of fixative and mix gently. Add fixative in this way until all cell clumps have dissolved and the cell suspension is as uniform as possible.
8. Centrifuge at 1500 rpm for 5 minutes.
9. Repeat steps 9-10 twice.
10. After the last wash, remove the supernatant as close to the pellet as possible and then resuspend in as much fixative as required for slide preparation.

Choice of method (by Rooney DE et al).
Several methods are possible. The choice of method depends greatly on the disease being investigated. Recommended treatment regimens are listed in the table below.

Diagnosis	Sample	Minimum Cultures	Extras
CGL/CML	PB/BM	ONC, S	D, 48h
AML (außer 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 5d + PMA; 2d + PHA	D, S, 24h, 48h
CLL (B cell)	PB/BM	ONC, 3d + PMA 5d + PMA; 2d + PHA	3d
CLL (T cell)	PB/BM	ONC, 3d + PHA 3d PMA	3d
T-cell lymphoma	LN/BM	ONC, 3d + PHA 3d + PMA	3d
B-cell lymphoma	LN/BM	ONC, 3d + PMA 5d + PMA	3d
Other lymphomatic diseases	BM	ONC, 3d +/- PMA 5d +/- PMA	S

APL: acute promyelocytic leukemia; MPD: myeloproliferative disorder; PB: peripheral blood; BM: bone marrow; LN: lymph nodes; ONC: overnight colcemid exposure; S: synchronized culture; PHA: phytohemagglutinin; PMA: 4-phorbol-12-myristate-13-acetate.

Important notes

- Occasionally, calcium oxalate crystals can form, but, to date, they have not shown any negative effects on cell growth.
- Thawing in a water bath at 37°C. should be avoided, as precipitates can form.

Warnings and precautions

- Maintaining the sterility of the product is necessary for its use, and this must be strictly adhered to by the user.
- Not suitable for therapeutic use with humans or animals.
- May only be used by trained, specialist personnel.
- Do not use bottles whose packaging has been damaged.
- Do not use CytogenMarrow Medium beyond its expiry date, which is stated on the label.
- The patient samples constitute biological material, and, therefore, safety precautions must be taken in accordance with local regulations for working with potentially infectious material.
- Cytogen GmbH does not guarantee the success of diagnostic testing solely through the use of Cytogen products.
- Report serious incidents that have occurred in connection with this product to the manufacturer and the competent authorities.

CE marking

With CytogenMarrow, Cytogen offers a CE-marked medium that meets the regulatory requirements for *in vitro* diagnostic medical devices set by the European Commission.

Explanation of symbols

	Observe instructions for use
IVD	*In vitro* diagnostic medical device
REF	Article number
LOT	Production lot number, batch
	Use by
	Temperature limit

	Manufacturer
	Date of manufacture
STERILE A	Sterilised using aseptic methods
	Do not use if the packaging is damaged
	Keep away from sunlight

Manufacturer

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