

CHANG Medium BMC

Bone Marrow Culture Medium

Catalog No. 91004
100mL, 500mL

 For *in vitro* diagnostic use.

 Zur *In-Vitro*-Diagnostik.

 Solo per uso diagnostico *in vitro*.

 Pour diagnostics *in vitro*.

 Para uso diagnóstico *in vitro*.

 Para utilização em diagnóstico *in vitro*.

 Για *in vitro* διαγνωστική χρήση.

 Uitsluitend voor *in vitro* diagnostisch gebruik.

 Pro diagnostické použití *in vitro*.

 Til *in vitro*-diagnostik.

 In *vitro*-diagnostiikkaan.

 Lietošanai *in vitro* diagnostikā.

 Pentru uz diagnostic *in vitro*.

 För *in vitro*-diagnostik.

 Do diagnostyki *in vitro*.

 In *vitro* diagnostiliseks kasutamiseks.

 In *vitro* diagnostikāi alkalmazáshoz.

 Skirta *in vitro* diagnostikai.

 In *vitro* diagnostik kullanim için.

 Na diagnostické použitie *in vitro*.

 За *in vitro* диагностична употреба.

 Za upotrebu u *in vitro* dijagnostici.

 Ghăl užu dijanjostiku *in vitro*.

 Za diagnostično uporabo *in vitro*.

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ENGLISH

INDICATION FOR USE

CHANG Medium BMC is intended for use in primary culture of clinical Human Bone Marrow Cultures for karyotyping and other genetic testing of various hematological disorders.

DEVICE DESCRIPTION

CHANG Medium BMC is a ready-to-use medium consisting of RPMI Medium 1640, with FBS, HEPES buffer, L-glutamine, Giant Cell Tumor (GCT) Conditioned Medium and Gentamicin Sulfate. CHANG Medium BMC has been optimized to support efficient cell attachment and growth of bone marrow cells for cytogenetic analysis. No addition of any components prior to culturing bone marrow is required.

COMPONENTS

<u>Amino Acid</u>	<u>Proteins,</u>	<u>Vitamins and trace</u>
Arginine	<u>Horomones, and</u>	<u>elements</u>
Asparagine	<u>Growth Factors</u>	Folic acid
Aspartic Acid	Fetal bovine serum	Nicotinamide
Cystine	(FBS)	Riboflavin
Glutamine		Thiamine
Glutamic Acid		Pantothenic acid
Glycine	<u>pH Indicator</u>	Cobalamin
Histidine	Phenol red	Pyridoxine
Hydroxyproline		Aminobenzoic acid
Isoleucine	<u>Salts & Ions</u>	
Leucine	Sodium chloride	<u>Other</u>
Lysine	Choline chloride	Giant cell tumor
Methionine	Potassium chloride	conditioned medium
Phenylalanine	Magnesium sulfate	(GCT-CM)
Proline	Sodium phosphate	Glutathione
Serine	Calcium Nitrate	Biotin
Threonine		
Tryptophan	<u>Buffers</u>	<u>Energy Substrates</u>
Tyrosine	Sodium bicarbonate	Glucose
Valine	HEPES	Inositol
	<u>Antibiotic</u>	
	Gentamicin Sulfate	
<u>Water</u>		
WFI Quality		

MATERIALS AND EQUIPMENT REQUIRED BUT NOT PROVIDED

1. Plastic Sterile Centrifuge Tubes and Culture Flasks
2. CO₂ Incubator at 37°C
3. Bench Centrifuge
4. Vortex Mixer
5. Colcemid Stock Solution, 10 µg/mL
6. Potassium Chloride Solution, 0.075 M
7. Fixative Solution, Methanol:Acetic Acid (3:1)

QUALITY ASSURANCE

Several factors including source of specimens, culture conditions and selection of reagents can influence the result obtained. Users are advised to run each new batch of reagent in parallel with reference material of known suitable activity before adoption in routine use. Each lot of CHANG Medium BMC has been performance tested on Clinical Bone Marrow Cultures at an independent Clinical Cytogenetics Laboratory compared to a control medium. Results are reported on a lot specific Certificate of Analysis.

PREPARATION FOR USE

CHANG Medium BMC should be thawed overnight in the refrigerator (2-8°C) then gently mixed to assure homogeneity. Aseptically dispense 10 mL of medium into sterile culture flasks and equilibrate to 37°C for immediate use for bone marrow cultures.

Note: Calcium carbonate crystals commonly form in CHANG Medium BMC. The presence of these crystals has not been shown to cause any detrimental effect on product performance.

DIRECTIONS FOR USE

Sample Preparation:

Use 0.5 to 1.0 mL of sodium heparinized bone marrow aspirate. Lithium heparin, EDTA, or citrate anticoagulants are unsuitable for cytogenetic studies.

- If more than 5 mL of bone marrow aspirate is received, the sample may be hemodilute. Spin the specimen down to isolate the bone marrow fraction.

- If the specimen arrives in transport medium, spin the sample down and remove the transport medium (supernatant). Inoculate using the remaining aspirate fraction.

For additional details on the use of these products, each laboratory should consult its own laboratory procedures and protocols which have been specifically developed and optimized for your individual medical program.

Bone Marrow Culture:

Label all culture vessels with patient name, specimen number, and culture type. For each specimen prepare a flask containing:

1. 10.0 mL CHANG Medium BMC.
2. Equilibrate flask to 37°C before inoculation of specimen.
3. Inoculate 0.5 mL (500 µL) of specimen, or the appropriate amount depending upon the white blood cell (WBC) count, into each flask containing 10.0 mL pre-equilibrated CHANG Medium BMC. Add less specimen if WBC is high (> 30,000) or more specimen if WBC is low (< 5,000).
4. Incubate flask at 37°C for 1-2 days.

Harvesting the Cultures:

1. Remove cultures from incubator and gently swirl to resuspend cells.
2. Transfer the contents of the flask to a 15 mL centrifuge tube.
3. Add 100 µL of stock Colcemid (10 µg/mL) to each tube.
4. Cap tubes and mix by inverting.
5. Incubate tubes at 37°C for 20 minutes.
6. After incubation, centrifuge tubes for 8 minutes at 1200 rpm (300 x g).
7. Carefully aspirate supernatant from each tube.
8. Resuspend the cell pellet by gently mixing, or flicking the bottom of the tube with forefinger.
9. VERY SLOWLY add 10 mL of hypotonic solution (0.075 M Potassium Chloride) to each tube while vortexing (on the lowest setting).
10. Let tubes stand at room temperature for 20 minutes (hypotonic treatment).
11. Centrifuge tubes for 8 minutes at 1200 rpm (300 x g).
12. Aspirate supernatant leaving about 1.0 mL of hypotonic solution above cell pellet.
NOTE: Be cautious of fibrous material that may extend from the cell pellet up into the supernatant after centrifugation. The last few mL of supernatant may need to be removed by hand with a Pasteur pipette (not using vacuum aspiration) to avoid aspirating the entire cell pellet into the waste container.
13. Resuspend cell pellet as described in step 8.
14. VERY SLOWLY add 10 mL of 3:1 Methanol:Acetic acid fixative to each tube while vortexing (on the lowest setting).
15. Let tubes stand at room temperature for 20 minutes (first fix).
16. Repeat steps 11 - 13.
17. Add 5 mL of fixative as in step 14.
18. Let tubes stand at room temperature for 10 minutes (second fix).
19. Repeat steps 16-18 (third fix).
20. At this point, fixed cell pellets can be used immediately for slide preparation according to the laboratory's standard protocol or stored in the refrigerator (2-8°C) for future use.

STORAGE AND STABILITY













CHANG Medium BMC should be stored frozen below -10°C until ready to use. CHANG Medium BMC is stable until the expiration date shown on the bottle label when stored frozen. After thawing, any unused product can be dispensed into working aliquots and refrozen for later use, or lightly capped and stored at 2°C to 8°C for up to 30 days. Protect from fluorescent light.

PRECAUTIONS AND WARNINGS

This device is intended to be used by staff trained in procedures that include the indicated application for which the device is intended.

CHANG Medium BMC contains FBS and GCT conditioned medium and should be handled with universal laboratory precautions. The medium contains an antibiotic (gentamicin) to reduce the potential for bacterial contamination, but aseptic techniques should always be used when dispensing the medium. Do not use any medium that is not red in color.

Glossary of Symbols*:

	Catalog Number
	Lot Number
	Sterilized using aseptic processing techniques (filtration)
	Expiration: Year - Month - Day
	Caution, consult accompanying documents
	Consult instructions for use
	Storage Temperature below -10°C
	Do not resterilize
	Do not use if package is damaged
	Manufacturer
	CE Mark
	Emergo Europe - Westervoorsdijk 60 6827 AT Arnhem The Netherlands

*Symbol Reference - EN ISO 15223-1, Medical devices - Symbols to be used with medical device labels, labeling.

INDIKACIJE ZA UPORABO

Medij CHANG Medium BMC je namenjen za uporabo v primarnih kliničnih kulturah humanega kostnega mozga za določanje kariotipa in druge genske preiskave različnih hematoloških motenj.

OPIS PRIPOMOČKA

Medij CHANG Medium BMC, ki je že pripravljen za uporabo in vsebuje RPMI Medium 1640, FBS, puffer HEPES, L-glutamin, Giant Cell Tumor (GCT) Conditioned Medium in gentamicinjev sulfat. Medij CHANG Medium BMC je optimiziran za podporo učinkovite pritrditve in rasti celic kostnega mozga za citogenetsko analizo. Pred gojenjem kostnega mozga ni treba dodati nobenih komponent.

KOMPONENTE

<u>Aminokislina</u>	<u>Beljakovine,</u>	<u>Vitaminski elementi</u>
Arginin	<u>hormoni in rastni</u>	<u>v sledovih</u>
Asparagin	<u>faktorji</u>	Folna kislina
Asparaginska kislina	Serum govejega zarodka (FBS)	Nikotinamid
Cistin		Riboflavin
Glutamin	<u>Indikator</u>	Tiamin
Glutaminska kislina	<u>vrstnosti pH</u>	Pantotenska kislina
Glicin	<u>Fenol rdeče</u>	Kobalamin
Histidin		Piridoksin
Hidroksiprolin	<u>Soli in ioni</u>	Aminobenzojska kislina
Izolevcin	Natrijev klorid	
Levcin	Holinoklorid	<u>Druga</u>
Lizin	Kalijev klorid	Kondicioniran medij iz velikoceličnih tumorjev (GCT-CM)
Melatonin	Magnezijev sulfat	Glutatin
Fenilalanin	Natrijev fosfat	Biotin
Prolin	Kalcijev nitrat	
Serin	<u>Putri</u>	<u>Energijski substrati</u>
Treonin	Natrijev bikarbonat	Glukoza
Triptofan	HEPES	Inozitol
Tirozin		
Valin	<u>Antibiotik</u>	
	Gentamicinjev sulfat	
<u>Voda</u>		
Kakovost,		
ki ustreza vodi za injekcije		

POTREBNI MATERIALI IN PRIPOMOČKI,**KI NISO PRILOŽENI**

1. Plastične, sterilne, celične epruvete in bučke za gojenje kultur
2. CO₂-inkubator s temperaturo 37 °C
3. Namizna centrifuga
4. Vrtnični mešalnik
5. Osnovna raztopina kolcemida, 10 µg/ml
6. Raztopina kalijevega klorida, 0,075 M
7. Fiksacijska raztopina metanola in ocetne kisline (razmerje 3 : 1)

ZAGOTAVLJANJE KAKOVOSTI

Na dobjeni rezultat lahko vpliva več dejavnikov, vključno z virom vzorcev, pogoji gojenja in izbiro reagentov. Uporabnikom svetujemo, da vsako novo serijo reagenta pred začetkom rutinske uporabe preskusijo v primerjavi z referenčnim materialom, za katerega je znana ustrezna aktivnost. Delovanje vsake serije medija CHANG Medium BMC je testirano na kliničnih kulturah kostnega mozga v neodvisnem laboratoriju za klinično citogenetiko v primerjavi s kontrolnim medijem. Rezultati so navedeni na analiznem certifikatu za vsako serijo.

PRIPRAVA ZA UPORABO

Medij CHANG Medium BMC je treba čez noč odtaliti v hladilniku (2–8 °C) in nato previdno premešati, da se zagotovi homogenost. Aseptično razdelite 10 ml medija v sterilne bučke za gojenje kultur in ga uravnotežite na 37 °C za takojšnjo uporabo s kulturami kostnega mozga.

Opomba: V mediju CHANG Medium BMC pogosto nastanejo kristali kalijevega karbonata, vendar prisotnost teh kristalov ni pokazala nobenih škodljivih učinkov na uporabnost izdelka.

NAVODILA ZA UPORABO

Priprava vzorcev:

Uporabile od 0,5 do 1,0 ml aspirata kostnega mozga z dodatkom natrijevega heparina. Litijev heparin, EDTA ali citratni antikoagulansi niso primerni za citogenetske študije.

- Če prejmete več kot 5 ml aspirata kostnega mozga, je vzrok lahko v hemodiluciji vzorca. V tem primeru vzorec centrifugirajte, da izolirate frakcijo kostnega mozga.

- Če vzorec prejmete v mediju za prenos, s centrifugiranjem odstranite medij za prenos (supernatant). Inokulirajte z uporabo preostale frakcije aspirata.

Dodatne podrobnosti o uporabi teh izdelkov določajo notranji laboratorijski postopki in protokoli vsakega laboratorija, ki so bili posebej razviti in optimizirani za zadevni medicinski program.

Gojitev kostnega mozga:

Na vse posode za gojenje kultur zapišite ime bolnika, število vzorca in tip kulture. Za vsak vzorec pripravite bučko, ki vsebuje:

1. 10,0 ml medija CHANG Medium BMC.
2. Pred inokulacijo vzorca bučko uravnotežite na 37 °C.
3. V vsako bučko, ki vsebuje 10,0 ml predhodno uravnoteženega medija CHANG Medium BMC, inokulirajte po 0,5 ml (500 µl) vzorca oziroma ustrezno količino glede na število belih krvnih celic (BKS). Če je število belih krvnih celic visoko (> 30.000), dodajte manj vzorca, in če je nizko (< 5000), dodajte več vzorca.
4. Bučko inkubirajte 1–2 dni pri temperaturi 37 °C.

Pobiranje kultur:

1. Kulture vzemite iz inkubatorja in jih nežno sukajte, da ponovno suspendirate celice.
2. Vsebinske bučke prenesite v 15 ml centrifugirno epruveto.
3. V vsako epruveto dodajte 100 µl osnovne raztopine kolcemida (10 µg/ml).
4. Epruvete zaprite in premešajte vsebino z obračanjem.
5. Epruvete 20 minut inkubirajte pri temperaturi 37 °C.
6. Po inkubaciji epruvete 8 minut centrifugirajte pri 1200 vrt./min (300 x g).
7. Previdno aspirirajte supernatant iz vsake epruvete.
8. Celično usedlino ponovno suspendirajte tako, da jo narahlo premešate ali s kazalcem frcate po spodnjem delu epruvete.
9. ZELO POČASI dodajte 10 ml hipotonične raztopine (0,075 M kalijevega klorida) v vsako epruveto med mešanjem v vrtničnem mešalniku (pri najnižji nastavitvi).
10. Epruvete naj 20 minut počivajo pri sobni temperaturi (hipotonična obdelava).
11. Epruvete 8 minut centrifugirajte pri 1200 vrt./min (300 x g).
12. Aspirirajte supernatant, tako da nad celično usedlino ostane približno 1,0 ml hipotonične raztopine. OPOMBA: Pazite na vlaknasto snov, ki se po centrifugiranju lahko širi iz celične usedline v supernatant. Zadnjih nekaj ml supernatanta boste morda morali ročno odstraniti s Pasteurjevo pipeto (ne z vakuumsko aspiracijo), da preprečite aspiracijo celotne celične usedline v posodo za odpadke.
13. Ponovno suspendirajte celično usedlino, kot je opisano v 8. koraku.
14. ZELO POČASI dodajte 10 ml fiksacijske raztopine metanola in ocetne kisline (razmerje 3 : 1) v vsako epruveto med mešanjem v vrtničnem mešalniku (pri najnižji nastavitvi).
15. Epruvete naj 20 minut počivajo pri sobni temperaturi (prvo fiksiranje).
16. Ponovite korake od 11 do 13.
17. Dodajte 5 ml fiksativa kot v 14. koraku.

18. Epruvete naj 10 minut počivajo pri sobni temperaturi (drugo fiksiranje).

19. Ponovite korake od 16 do 18 (tretje fiksiranje).

20. Na tej točki se lahko fiksirani celični peleti takoj uporabijo za pripravo preparatov skladno s standardnim protokolom laboratorija ali shranijo v hladilnik (2–8 °C) za nadaljnjo uporabo.

SHRANJEVANJE IN STABILNOST

Medij CHANG Medium BMC je treba shranjevati zamrznjen pri temperaturi pod –10 °C, dokler ni pripravljen za uporabo. Če se medij CHANG Medium BMC shranjuje zamrznjen, je stabilen do datuma izteka roka uporabnosti, ki je naveden na nalepki steklenice. Odtaljen izdelek, ki ga niste porabili, lahko razdelite na delovne alikote in ponovno zamrznete za poznejšo uporabo ali pa dobro zaprete s pokrovčkom in do 30 dni hranite pri temperaturi 2–8 °C. Zaščitejte pred fluorescenčno svetlobo.

PREVIDNOSTNI UKREPI IN OPOZORIILA

Ta pripomoček sme uporabljati samo osebno, usposobljeno za postopke, ki vključujejo indiciranje uporabo, za katero je pripomoček zasnovan.

CHANG Medium BMC vsebuje kondicioniran medij (FBS in GCT) in z njim je treba ravnati ob upoštevanju univerzalnih laboratorijskih previdnostnih ukrepov. Medij vsebuje antibiotik (gentamicin) za zmanjšanje tveganja bakterijske kontaminacije, vendar je treba pri razporejanju medija vedno uporabljati aseptične tehnike. Ne uporabljajte nobenega medija, ki ni rdeče barve.