TIMERS

"Clip" and "Onehand" models have mechanical movement, with timing up to 60 min. "Electronic" model is battery operated, has a 4-digit display up to 19 hours 59 minutes, an electronic alarm and a free-standing clip and magnetic support. Material: ABS case.

Cod.	Dim. mm	Colour	Mod.
10853	50x62x23	White	Electronic

Digitally signed by Ceaicovschi Tudor
Date: 2022.11.22 10:14:13 EET
Reason: MoldSign Signature
Location: Moldova

CERTIFICATO N° 505SGQ05

CERTIFICATE N° 505SGQ05

Si certifica che il
this is to certify that

Sistema di Gestione per la Qualità

Quality Management System

messo in atto da
implemented by

APTACA S.p.A.

Via Monte Bianco, 4 – IT 20900 MONZA (MB)

nella Sede Operativa di
Operative Unit

Regione Monforte, 30 – IT 14053 CANELLI (AT)

è conforme alla norma
is in compliance with the standard

UNI EN ISO 9001-2015 (ISO 9001-2015)

per i seguenti Processi
concerning the following kinds of Processes

Gestione della fabbricazione ed immissione in commercio di tamponi sterili per il prelievo di campioni biologici in orifizi naturali e in ambito chirurgico. Progettazione e fabbricazione di dispositivi medico diagnostici per laboratori di analisi. Gestione della fabbricazione ed immissione in commercio di dispositivi medici invasivi in relazione agli orifizi del corpo in Classe I Sterile. Fabbricazione di dispositivi medici invasivi in relazione agli orifizi del corpo in Classe I Sterile. Commercializzazione di dispositivi medici e diagnostici in vitro. Commercializzazione di articoli da laboratorio

Management of the manufacturing and placing on the market of sterile tampons for sampling of biological specimens in natural orifice and in surgical field. Design and manufacturing of diagnostic medical devices for laboratories of analysis. Management of the manufacturing and placing on the market of invasive medical devices with respect to body orifices (class I sterile). Manufacturing of invasive medical devices with respect to body orifices (class I sterile). Marketing of medical and diagnostic devices in vitro. Marketing of laboratory articles.

Il presente Certificato è soggetto al rispetto delle condizioni stabilite dai Regolamenti per la certificazione in vigore applicabili.
This Certificate shall satisfy the requirements established in the Rules for the certification in force applicable.
In caso di discordanza tra le lingue utilizzate nella traduzione del contenuto del presente certificato, fare riferimento alla lingua italiana
In cases of discrepancy between the languages used in the translation of the content of this certificate, please refer to the Italian language

L'AMMINISTRATORE DELEGATO
MANAGING DIRECTOR

Dr. Ing. Roberto Cusolito

Data di Prima Emissione *First Issue Date*	Data di Prima Emissione ITALCERT *First Issue Date ITALCERT*	Data di Rinnovo *Renewal Date*	Data di Scadenza *Expiration Date*
1998-07-23	2011-10-30	2020-10-30	2023-10-29

Settore IAF 14 - 29

SGQ N° 023A
Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
Signatory of EA, IAF and ILAC Mutual Recognition Agreements

ITALCERT S.r.l. | Viale Sarca, 336 – 20126 Milano (MI) | tel. +39 0266104876 | fax. +39 0266101479 | www.italcert.it | italcertsrl@legalmail.it

CERTIFICATO N° 505DM07

CERTIFICATE N° 505DM07

Si certifica che il
this is to certify that

Sistema di Gestione per la Qualità

Quality Management System

messo in atto da
implemented by

APTACA S.p.A.

Via Monte Bianco, 4 – IT 20900 MONZA (MB)

nella Sede Operativa di
Operative Unit

Regione Monforte, 30 – IT 14053 CANELLI (AT)

è conforme alla norma
is in compliance with the standard

UNI CEI EN ISO 13485-2016 (ISO 13485-2016)

per i seguenti Processi
concerning the following kinds of Processes

Gestione della fabbricazione e immissione in commercio di tamponi sterili per il prelievo di campioni biologici in orifizi naturali e in ambito chirurgico. Progettazione e fabbricazione di dispositivi medico diagnostici per laboratori di analisi. Gestione della fabbricazione ed immissione in commercio di dispositivi medici invasivi in relazione agli orifizi del corpo in Classe I Sterile. Fabbricazione di dispositivi medici invasivi in relazione agli orifizi del corpo in Classe I Sterile. Commercializzazione di dispositivi medici e diagnostici in vitro.

Management of the manufacturing and placing on the market of sterile tampons for sampling of biological specimens in natural orifice and in surgical field. Design and manufacturing of diagnostic medical devices for laboratories of analysis. Management of the manufacturing and placing on the market of invasive medical devices with respect to body orifices (class I sterile). Manufacturing of invasive medical devices with respect to body orifices (class I sterile). Marketing of medical and diagnostic devices in vitro.

Il presente Certificato è soggetto al rispetto delle condizioni stabilite dai Regolamenti per la certificazione in vigore applicabili.
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Data di Prima Emissione *First Issue Date*	Data di Prima Emissione ITALCERT *First Issue Date ITALCERT*	Data di Rinnovo *Renewal Date*	Data di Scadenza *Expiration Date*
2007-10-30	2011-10-30	2020-10-30	2023-10-29

SGQ N° 023A
Membro degli Accordi di Mutuo Riconoscimento EA, IAF e ILAC
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ITALCERT S.r.l. | Viale Sarca, 336 – 20126 Milano (MI) | tel. +39 0266104876 | fax. +39 0266101479 | www.italcert.it | italcertsrl@legalmail.it

EC Certificate

TÜVRheinland®

Production Quality Assurance
MDD Annex V

Registration No.: DD 2063008-1

Manufacturer: Boen Healthcare Co., Ltd.
Unit 602, International Center, No. 535, Shenxu Road,
Suzhou,
215021 Jiangsu
P.R. China

Medical Brushes, Disposable Vaginal Speculums, Disposable Gynecological Sets,
Disposable Dressing Kits, Disposable Colostomy Bags, Disposable Umbilical Cord Clamps, Disposable Urine Drainage Bags, Sterile Wooden Tongue Depressors, Non Woven Surgical Drapes, Non Woven Surgical Gowns, X-ray Detectable Gauze Swabs (Sponges), Gauze Balls and Lap Sponges in Sterilization Packing, Gauze Swabs (Sponges), Gauze Balls Gauze Bandages and Non Woven Wound Care Products, Medical Elastic Bandages, First Aid Kits and Its Related Products, Disposable Nasal Speculums, Disposable Ear Checkers, Disposable Oral Cavity Kits and Implements, Sterile Urine Meters;
Aspects of manufacture concerned with conformity of products with metrological requirements: Sphygmomanometers, Mercury-free Clinical Thermometers

Replaces Approval, Registration No.: DD 60142274 0001

Report No.: 15092074 009

Effective date: 2020-11-18

Expiry date: 2024-05-26

Issue date: 2020-11-18

TÜV Rheinland LGA Products GmbH

Jason Pan
TÜV Rheinland LGA Products GmbH
Tillystraße 2 · 90431 Nürnberg · Germany

TÜV Rheinland LGA Products GmbH is a Notified Body according to Directive 93/42/EEC concerning medical devices with the identification number 0197.

EG-KONFORMITÄTSERKLÄRUNG · EC DECLARATION OF CONFORMITY
DÉCLARATION CE DE CONFORMITÉ · DICHIARAZIONE CE DI CONFORMITÀ

Name und Adresse des Herstellers: / Name and address of the manufacturer: / Nom et adresse du fabricant: / Nome e indirizzo del fabbricante:	**BOEN HEALTHCARE CO., LTD Unit 602, International Center, No.535, Shenxu Road, Suzhou, 215021, Jiangsu, China**

Wir erklären in alleiniger Verantwortung, dass / We declare under our sole responsibility that / Nous déclarons sous notre propre responsabilité que / Dichiariamo sotto la sola responsabilità che

das Medizinprodukt: / the medical device: / le dispositif médical: / il dispositivo medico:	**Gilson Pipette Tips**
der Klasse: / of class: / de la classe: / di classe:	**Common/Others IVD (Devices of NOT Annex II and NOT self-test)**

(IVDD, Artikel 9 Absatz 1) nicht Teil der Liste A und B von Anhang II sein / (IVDD, Article9(1)) not be part of list A & B of annex II (IVDD, article 9, paragraphe 1) ne fait pas partie de la liste A et B de l'annexe II / (IVDD, articolo 9, paragrafo 1) non fanno parte dell'elenco A e B dell'allegato II

den einschlägigen Bestimmungen der Medizinprodukte-Richtlinie 98/79/EG und deren Umsetzungen in nationale Gesetze entspricht. Die Erklärung gilt in Verbindung mit dem zum Produkt gehörigen „Endprüfprotokoll“. /

meets the provisions of the directive 98/79/EC and its transpositions in national laws which apply to it. The declaration is valid in connection with the “final inspection report” of the device. /

remplit toutes les exigences de la directive sur les dispositifs médicaux 98/79/EC et de ses transpositions en droit national qui le concernent. La déclaration est valable si elle est associée au «rapport de l’inspection finale» du produit. /

soddisfa tutte le disposizioni della direttiva 98/79/EC e della loro trasposizione nel diritto nazionale che lo riguardano. Questa dichiarazione è valida in congiunzione con il “rapporto di ispezione finale” del prodotto.

Konformitätsbewertungsverfahren: / Conformity assessment procedure: / Procédure d’évaluation de la conformité: / Procedura di valutazione della conformità:	**Anhang III (voraussichtlicher Punkt 6) der IVDD 98/79 / EG Annex III (expect point 6) of IVDD 98/79/EC Annexe III (sauf le point 6) de l'IVDD 98/79 / CE Allegato III (aspettarsi il punto 6) dell'IVDD 98/79 / CE**
Registrier-Nr.: / Registration No.: / N°d’enregistrement: / Numero di registrazione:	
Benannte Stelle: / Notified Body: / Organisme notifié: / Organismo notificato:	

CE

Suzhou, 201.05.26

General Manager

Ort, Datum / Place, date / Lieu, date / Luogo, data	Name und Funktion / Name and function / Nom et fonction / Nome e funzione

LABSYSTEMS DIAGNOSTICS
speaking your language

NeoNat SCID SMA

REAL TIME PCR KIT

The NeoNat SCID SMA is a Real-Time PCR assay for screening Severe Combined Immunodeficiency (SCID) Syndrome and Spinal Muscular Atrophy (SMA) in newborns DNA from Dried Blood Spot (DBS) samples.

- Greater flexibility with separate multiplexing of SCID and SMA from one kit
- Kit contains all the reagents and components to perform DNA extraction from DBS
- Ready to use reaction mix and calibrators preloaded plates enable convenience for users
- Four levels of DBS controls with defined target gene copies per µL
- Compatible with multiple qPCR instruments
- Short turnaround time of ~120 mins from DNA extraction to result analysis

CE IVD RUO Research Use Only

Assay Description

- NeoNat SCID SMA Real time PCR kit is based on 5' nuclease technique
- Screens T-cell receptor excision circle (TREC) / Kappa deleting recombination excision circle (KREC) for SCID and Survival Motor Neuron 1 (SMN1) & Survival Motor Neuron 2 (SMN2) for SMA. The β-globin gene serves as an internal control

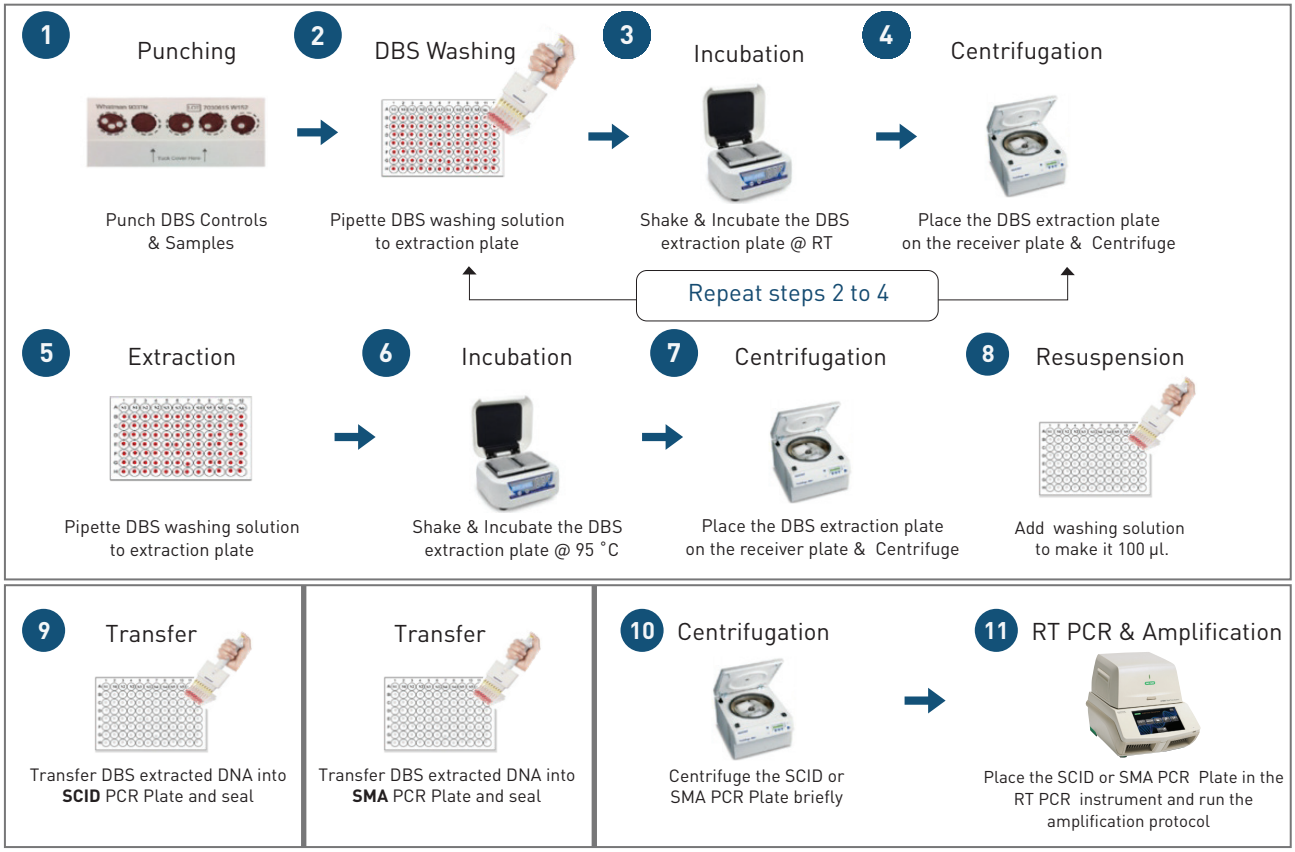
Performance

- The assay demonstrates excellent performance with 100% sensitivity and specificity for all the samples tested
- The analytical sensitivity shows a limit of detection of <5.0 copies/µL for TREC & KREC and <2.5 copies/µL for SMN1 & SMN2

Compatibility

- Kit compatible with most real-time PCR instruments in the market with at least three measurement channels (FAM, VIC/or HEX, & CY5)
- Developed and validated with Biorad CFX 96 and Thermofisher Quantstudio 5 & 6.

DNA EXTRACTION FROM DBS & PCR REACTION



Ordering Information

96 Reactions	8100481
192 Reactions*	8100482
480 Reactions*	8100483

*Please check the availability

For more information please order your IFU from
Labsystems Diagnostics Oy
Tiilitie 3, FI-01720 VANTAA, Finland Tel: +358 (0) 20 155 7530
sales@labsystemsdx.com | www.labsystemsdx.com

Declaration of Conformity

LABSYSTEMS DIAGNOSTICS
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Identification of the legal entity	**Labsystems Diagnostics Oy** **Tiilitie 3, 01720 Vantaa** **Finland** **Tel. +358-(0)20 155 7530** **Fax. +358-(0)20 155 7521**
Identification of the device(s) concerned	**NeoNat SCID-SMA Real-Time PCR Kit** *cat. no. :* 8100481

We hereby declare that the above mentioned device complies with the requirements of Council Directive 98/79/EC and the corresponding Finnish National Act 629/2010 and to the following standards.

Standards	**EN ISO 14971:2019** *Medical devices- application of risk management to medical devices.* ***EN ISO 15223-1:2016*** *Medical devices. Symbols to be used with medical device labels, labelling and information to be supplied. Part 1: General requirements.* ***EN ISO 13485:2016*** Medical devices. Quality management systems. Requirements for regulatory purposes. **EN 13612:2002** Performance evaluation of *in vitro* diagnostic medical devices **EN ISO 23640:2015** *In vitro* diagnostic medical devices. Evaluation of stability of *in vitro* diagnostic reagents **EN ISO 18113-1:2011** *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) Part 1: Terms, definitions and general requirements

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Declaration of Conformity

	EN ISO 18113-2:2011 *In vitro* diagnostic medical devices - Information supplied by the manufacturer (labelling) Part 2: In vitro diagnostic reagents for professional use
IVDD classification	**General IVDD**
Conformity assessment procedure	**Annex III of the Directive 98/79/EC**
Notified body	**Not applicable**
Signature of the authorized person	In Vantaa on the 13th April 2021 **Sameer Saral** **Chief Operations Officer**

www.labsystemsdx.com
LABSYSTEMS DIAGNOSTICS OY
VANTAA | FINLAND

Labsystems Diagnostics Oy
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LABSYSTEMS DIAGNOSTICS
speaking your language

REF 8100481, 8100482, 8100483

NeoNat SCID-SMA

Real-Time PCR Kit

CE

96/192/480 reactions

Instructions for use

IVD

Labsystems Diagnostics Oy
Tiilitie 3, FI-01720 Vantaa, Finland
Tel. +358-20-155 7530, Fax +358-20-155 7521
E-mail: contact@labsystemsdx.com
www.labsystemsdx.com

25.05.2022

INSTRUCTION FOR USE

The NeoNat SCID-SMA Real-Time PCR Kit is a Real-Time PCR assay for screening Severe Combined Immunodeficiency (SCID), X-linked Agammaglobulinemia (XLA) and Spinal Muscular Atrophy (SMA) in newborn's DNA from Dried Blood Spot (DBS) samples.

Product no.:

8100481 .....96 reactions

8100482.....192 reactions

8100483 ..... 480 reactions

CONTENTS Page

INTENDED USE

Labsystems Diagnostics' NeoNat SCID-SMA Real-Time PCR Kit is a Real-Time PCR kit for the screening of Severe Combined Immunodeficiency (SCID) by semi quantitative determination of *TREC* (T-cell Receptor Excision Circle), & *KREC* (Kappa-deleting Recombination Excision Circle), Spinal Muscular Atrophy (SMA) by measuring *SMN1* (Survival Motor Neuron 1), & *SMN2* (Survival Motor Neuron 2) and X-linked agammaglobulinemia (XLA) in newborn's DNA extracted from DBS samples.

This assay should only be used in conjunction with other clinical and confirmatory laboratory findings and results from this test alone should not be used to make diagnostic or treatment decisions.

INTRODUCTION

Severe Combined Immunodeficiency

Severe combined immunodeficiency is a group of rare disorders caused by mutations in different genes involved in the development and function of infection-fighting immune cells. Patients with SCID have low numbers of T-cells and their B-cells do not function. Most often, SCID is inherited in an autosomal recessive pattern, in which both copies of a particular gene - one inherited from the mother and one from the father - contain defects. X-linked SCID, which is caused by mutations in a gene on the X chromosome, primarily affects male infants [1, 2].

The SCID newborn screening test measures *TREC*s, a byproduct of T-cell development. *TREC*s are small pieces of DNA generated in T-cells as they mature. Because infants with SCID have few or no T-cells, the absence of *TREC*s may indicate SCID. Research supported by National Institute of Health and other organizations has shown that early diagnosis of SCID through newborn screening leads to prompt treatment and high survival rates [1, 2].

Molecular assay that detects the absence of *TREC*s using DBS samples has been the most effective way to diagnose SCID. There are other genetic syndromes (e.g., DiGeorge) or conditions (e.g., congenital heart disease) that can also lead to reduction in the number of circulating T-cells [1-4].

KRECs, an extrachromosomal excision products similar to *TRECs*, are formed as a result of generation of diverse B-cell receptor light chains. A rise in *KREC*s reflects the presence of newly derived bone marrow B-cells after hematopoietic cell transplantation in primary immune deficient patients. *KREC* levels in DNA isolated from human blood are undetectable in primary immunodeficiencies (PIDs) in which B-cells are absent or dysfunctional. Thus, testing newborn DBS samples for the presence of *KREC*s is potentially useful for identifying neonates with defects in early B-cell maturation. *TREC*s and *KREC*s can be measured simultaneously with Labsystems Diagnostics' NeoNat SCID-SMA Real-Time PCR Kit [5, 6].

To confirm a SCID diagnosis, a doctor will evaluate the numbers and types of T-cells and B-cells present in the blood samples as well as their ability to function [7].

X-linked Agammaglobulinemia

X-linked agammaglobulinemia (XLA) is one of the PIDs caused by mutations in Bruton tyrosine kinase (BTK) or B-cell negative SCID. XLA is characterized by severe B-cell lymphopenia and marked reduction in all classes of serum immunoglobulins. XLA can be identified by screening for *KREC*, the excised circular by-product of B-cell immunoglobulin kappa gene rearrangement [5-7].

Spinal Muscular Atrophy

Spinal muscular atrophy (SMA) is a group of hereditary diseases that are caused by progressive degeneration of motor neurons - nerve cells in the brain stem and spinal cord, which control essential skeletal muscle activity and important body functions such as speaking, walking, breathing, and swallowing. The death of motor neurons leads to muscle weakness and atrophy [8, 9].

The disorder is caused by defects in both copies of the survival motor neuron 1 gene (*SMN1*) on chromosome 5q. This gene produces the survival motor neuron (SMN) protein which maintains the health and normal function of motor neurons. Low levels of SMN1 protein result in loss of function of neuronal cells in the spinal cord which in turn leads to weakness and degeneration (or atrophy) of the skeletal muscles [8-11].

There are two *SMN* genes, *SMN1* and *SMN2, which are* nearly identical and encode the same protein. However, *SMN2 gene* contains a mutation that disrupts a splice enhancer resulting in production of mainly unstable and less functional protein. Only 10-20% of SMN protein synthesized from *SMN2* gene are fully functional.

Mutations in *SMN1* are associated with spinal muscular atrophy; whereas mutations in *SMN2*, do not lead to SMA. Simultaneous detection of SMA-related mutations in both *SMN1* and *SMN2* by PCR with specific primers and probes, is more reliable way of screening for SMA, than detecting only *SMN1* mutations, because the multiplication of *SMN2* gene can compensate to some extent the negative effect of *SMN1* loss-of-function mutations. To treat SMA, SMA clinical working group unanimously recommended immediate treatment for individuals predicted to manifest SMA with the qualifying genotypes of two or three copies of *SMN2* as supported by the strong positive results arising from in the NURTURE trial [8,12].

PRINCIPLE OF THE TEST

The principle of the Labsystems Diagnostics' NeoNat SCID-SMA Real-Time PCR kit is based on the 5' nuclease amplification technique. NeoNat *SCID-SMA* Real-Time PCR kit is designed to measure *TREC, KREC*, *SMN1, SMN2 and β-globin (BG)* genes from DBS samples of newborns. The kit includes 2 plates with ready to use optimized reaction mixture for simultaneous amplification and detection of 3 genes per plate: (i) *TREC, KREC* and *β-globin* genes in SCID PCR reaction (*β-globin* is used as an internal control) and (ii) *SMN1, SMN2* and *β-globin* genes in SMA PCR reaction (*β-globin* is used as an internal control). The following steps are carried out:

1. A 3.2 mm disk is punched out from Newborn DBS specimens and DBS controls.
2. The DNA is eluted from Newborn DBS specimens and DBS control discs.
3. The DNA samples from Newborn DBS specimens and controls are added to ready to use PCR plate which contains all the reagents necessary to run the assay.
4. The PCR plate is transferred to Real-Time PCR instrument for amplification.
5. The fluorescence signal from target specific probes is measured using corresponding channels.

(Optional: procedures listed above are also performed with DBS calibrators when they are used).

This kit is validated in CFX96 Real-Time PCR instrument from Bio-Rad Laboratories and the compatability of this SCID-SMA Real-Time PCR kit is confirmed with ABI QuantStudio™ 5 Real-Time PCR System. It can be also used with other Real-Time PCR instrument with minimum of three measurement channels (FAM, HEX/VIC and CY5). The quality control criteria provided in the IFU can deviate from the user's data acquired using their own PCR instrument. Kit contains passive reference dye that is detected using the ROX channel. The threshold cycle (C_t) values are calculated after baseline correction. The result interpretation for each condition is based on C_t values of three genes (*TREC, KREC* and *β-globin for* SCID and XLA*; SMN1,SMN2 and β-globin for* SMA*)*. The test performance is monitored by four level DBS controls.
If the kit is used with other commercially available equipments, the methods shall be verified and validated as appropriate.

SCID PCR Assay

Gene	Detection Channel
TREC	FAM
KREC	VIC/HEX
β-globin	CY5
Optional ROX background correction	

In SCID PCR amplification plate, the amplification of *TREC* is measured with the FAM fluorescence channel, the amplification of *KREC* is measured with the VIC/HEX fluorescence channel and the amplification of the internal control is measured with the CY5 fluorescence channel. Depending on the qPCR instrument used, ROX channel can be used for background correction.

SMA PCR Assay

Gene	Detection Channel
SMN1	FAM
SMN2	VIC/HEX
β-globin	CY5
Optional ROX background correction	

In the SMA PCR amplification plate, the amplification of *SMN1* is measured with the FAM fluorescence channel, the amplification of *SMN2* is measured with the VIC/HEX fluorescence channel and the amplification of the internal control is measured with the CY5 fluorescence channel. Depending on the qPCR instrument used, ROX channel can be used for background for correction.

KIT CONTENTS

Note:

1. **Storage:** PCR plates and DBS calibrator and DBS controls (kit box 2) must be stored at -20 °C (-18°C - -24°C). Other reagents (kit box 1) must be stored at room temperature (RT) between +10 °C and +27 °C
2. **Shelf life**: The expiration date is printed on each component's label and on the package. Do not use reagents after the expiration date.
3. Protect all the kit components from moisture and exposure to light.

I. SCID-SMA Real-Time PCR kit Box 1 (Shipped at **RT (+10 °C - +27 °C)**)

S. No.	Content	96 Rxn/kit	192 Rxn/kit	480 Rxn/kit
1	DBS Extraction plate	1 pc	2 pcs	5 pcs
2	DBS Extraction plate lid	1 pc	2 pcs	5pcs
3	Receiver plate	1 pc	2 pcs	5 pcs
4	Holding clip	2 pc	2 pcs	2 pcs
5	96 well plate	1 pc	2 pcs	5 pcs
6	DBS Washing solution	1x60ml	2x60ml	5x60ml
7	DBS Extraction solution	1x15ml	1x30ml	1x75ml
8	Adhesive PCR plate seal	4 pcs	8 pcs	20 pcs
9	Reagent Basin	3 pcs	6 pcs	15 pcs
10	IFU	1	1	1

II. SCID-SMA Real-Time PCR kit Box 2 (To be either shipped with **dry ice or by maintaining at -20 °C (-18°C - -24°C)).**

1. **SCID PCR plate 1/2/5 pcs**

Contains dNTPs, $MgCl_2$, PCR buffer, specific primers and probes and DNA polymerase needed for the detection and quantification of *TREC*, *KREC,* and *β-globin*. **Row A (A1-A12) contains the mixture of *TREC, KREC, and* *β-globin* plasmid DNA in duplicates in concentrations from 1,000,000 to 10 copies per µl for each target (see plate layout below)*.** The data collected from these wells are used for preparing a standard curve for SCID assay.

2. SMA PCR plate 1/2/5 pcs

Contains dNTPs, $MgCl_2$, PCR buffer, specific primers and probes and DNA polymerase needed for the detection and quantification of *SMN1, SMN2, and β-globin*. **Row A (A1 -A12) contains the mixture of *SMN1, SMN2, and β-globin* plasmid DNA in duplicates in concentrations from 1,000,000 to 10 copies per µl for each target (see plate layout below)*.** The data collected from these wells are used for preparing a standard curve for SMA assay.

3. DBS Control 1/2/5 pcs **CONTROL**

The DBS control panel includes 4 different DBS spots which contain defined number of copies of *TREC, KREC, SMN1, SMN2* and *β-globin* plasmid DNA per µl of blood in the DBS. The approx. number of copies per µl of *TREC, KREC, SMN1, SMN2* and *β-globin* in DBS controls are given in the section **QUALITY CONTROL**. The place of the DBS controls on the plate are marked with C1-C4 in the plate layout table below.

4. DBS Calibrator 1/2/5 pcs **CAL**

Optional: The DBS calibrator panel includes 4 different DBS spots (Cal 1, Cal 2, Cal 3, and Cal 4) which contain TREC, KREC, SMN1, SMN2 and β-globin plasmid DNA in defined concentrations (in copies per µl of the blood in the DBS). The use of DBS calibrators is optional. The data collected from the wells containing DBS calibrators can be used as alternative to prepare standard curve. If DBS calibrators are used to prepare standard curve, the lab should establish their own standard curve acceptance criteria and assay cut-off.

Well Positions A1-12 in PCR plate already contain all the reagents required for the standard curve preparation

Plate Format

	1	2	3	4	5	6	7	8	9	10	11	12
A	A	A	B	B	C	C	D	D	E	E	F	F
B	C1	C1	C2	C2	C3	C3	C4	C4				
C												
D												
E												
F												
G												
H												

Wells	Marked above as	Plasmid Calibrators
A1-A2	A A	10^6 copies/µl
A3-A4	B B	10^5 copies/µl
A5-A6	C C	10^4 copies/µl
A7-A8	D D	10^3 copies/µl
A9-A10	E E	10^2 copies/µl
A11-A12	F F	10^1 copies/µl

MATERIALS REQUIRED BUT NOT PROVIDED WITH KIT

1. Real-Time PCR instrument with at least three detection channels suitable for FAM, HEX/VIC, and CY5 measurement.
2. Disposable gloves.
3. Adjustable pipettes (20-200 µl, 1-10 µl).
4. Sterile pipette tips with filter (10 µl, 40-200 µl).
5. 96-well plate centrifuge (Any plate centrifuge that can provide the speed of 2500 rpm).
6. A 96-well plate shaking incubator with temperature control of at least 99 °C and shaking up to 1000 rpm.
7. DBS puncher
8. Tweezers

SPECIMEN COLLECTION AND HANDLING

1. Whole blood from newborn heel prick must be collected on Guthrie card with suitable filter paper and dried to form DBS. Collection of samples must be done as DBS from infants for newborn screening following CLSI document LA4-A5 [13-15].
2. The DBS must be dried in clean, dry and protected area for at least 3 hrs or overnight at RT.
3. Each DBS card must be packed separately in a bag with desiccant to prevent contamination from each other.
4. Newborn screening DBS specimens should be tested upon receipt and can be stored at ambient temperature with low humidity during handling.
5. The DBS samples are punched in 3.2 mm in diameter discs to the filter plate for DNA extraction.
6. Making a blank punch (punching in an empty filter paper) in between the samples is needed to avoid cross contamination of the DBS samples.
7. Low humidity and low temperatures (+4 - +8 °C) are suggested for short term storage of DBS. For storage of more than 2 years, low humidity and frozen conditions (-20 to -70 °C) are recommended.

PRECAUTIONS

Warning - POTENTIAL BIOHAZARDOUS MATERIAL:

All human materials used in the preparation of the calibrators/controls in the kit have been tested for the presence of the antibodies to HIV (Human Immunodeficiency Virus) and HCV (Hepatitis C Virus) as well as Hepatitis B surface antigen (HBsAg) and found to be non-reactive. As no test method can offer complete assurance that HIV, hepatitis B virus, HCV, or other infectious agents are absent, DBS calibrators, DBS controls and Newborn DBS samples should be handled at the Biosafety level 2 as recommended for any potentially infectious human serum or blood sample in the Centers for Disease Control and Prevention/National Institutes for Health Manual, “Biosafety in Microbiological and Biomedical Laboratories”, 5th Ed. 2009.

Discard all materials and samples as if capable of transmitting infection. The preferred method of disposal is autoclaving for a minimum of one hour at +121°C. Liquid wastes not containing acid and neutralized waste may be mixed with sodium hypochlorite in volumes such that the final mixture contains 50-500 mg/l free chlorine. Allow 30 minutes for decontamination to be completed. Spills should be wiped off thoroughly using either an iodophor disinfectant or sodium hypochlorite solution. Materials used to wipe off spills should be added to biohazardous waste matter for proper disposal. Reusable glassware must be disinfected, washed and rinsed free of detergents.

1. Avoid contact with skin and eyes when handling extraction and elution solutions.
2. Extreme precautions should be taken to prevent contamination of the reactions. Amplification procedures require highly skilled professionals.
3. Tubes containing any clinical samples and PCR plates should never be opened at the same time.
4. If the kit contents are broken/leaking, the kit components should not be used.
5. Wear disposable gloves while handling samples and kit reagents. Afterwards wash hands carefully. Never pipette by mouth.
6. Do not use the same pipette tips to handle different samples.

7. All pipettes used must be equipped with sterile filter tips.
8. Do not re-use a strip or plate even if some wells were not used.
9. Accurate and precise pipetting, as well as following the exact time and temperature requirements, are essential.
10. Do not eat, drink or smoke in dedicated work areas.
11. Disposal of all waste should be in accordance with local regulations.
12. All testing labs should strictly adhere to CLSI or equivalent regulatory guidelines [16,17]

TEST PROCEDURE

Please read the test procedure carefully before starting the experiement

DBS washing and extraction

1. **Keep row A (A1-A12) of the extraction plate empty**
2. Punch 3.2 mm disks from DBS controls (C1, C2, C3, and C4) in duplicates to wells B1-B8.
   Optional: punch 3.2 mm disks from DBS Calibrators in duplicates into the wells of 96 well DBS extraction plate.
3. Then punch 3.2 mm DBS clinical samples into wells from B9 to H12.
4. Pipette 200 µl of DBS washing solution to each well ***except wells in row A (A1-A12).*** **Keep row A (A1-A12) empty.** ***Use the plate cover to avoid contamination and evaporation of samples***.
5. Incubate the DBS extraction plate in a shaker incubator at RT at 700 rpm for 10 minutes.
6. After incubation, stack the DBS extraction plate on the receiver plate and fix them tight with the clip. Then centrifuge at 2500 rpm for 2 minutes and discard the flow through.
7. Repeat steps 4-6.
8. Pipette 65 µl of DBS extraction solution to each well **except wells in row A (A1-A12). Keep row A (A1-A12) empty.** ***Cover the plate with the plate cover to avoid contamination and evaporation of samples.***
9. Incubate the DBS extraction plate in a shaker incubator at +95 °C with shaking at 700 rpm for 15 minutes.
10. After incubation, cool the DBS extraction plate at RT for 2 minutes, place on top of a fresh 96-well plate and i**mmediately** centrifuge the sample at 2500 rpm for 2-3 minutes.
11. The volume of extracted DNA solution is ~50 µl. It is possible that some of the DBS spot extracted DNA is not filtered through the extraction plate to the 96 well plate. In that case, the extracted DNA in the DBS extraction plate can be pippetted out manually to the 96 well plate.
12. Add 50 µl of DBS washing solution to the ~50 µl of extracted DNA solution ***except wells in row A (A1-A12).*** **Keep row A (A1-A12) empty.** Mix it thoroughly by brief shaking the plate in the plate shaker. The total volume of DNA solution is approx.100 µl.

PCR amplification

13. Remove the PCR plates from the freezer and leave at RT for 5 minutes to thaw. Centrifuge briefly at 1500 rpm for 30 seconds.
14. **Leave the seal for A row (A1-A12) unopened.** ***Row A (A1-A12) is already loaded with plasmid calibrators.*** **There is a cut line at the seal between rows A & B. Carefully remove the aluminium seal from wells B1 to H12.**
15. Transfer 10 µl of the DNA solution from the plate containing extracted DNA to the wells B1-H12 of PCR plate. **Do not add anything to the wells in row A** ***(wells A1-A12 are pre-loaded with plasmid calibrators).***
16. Seal the wells from B1 to H12 with plastic PCR sealer.
17. Carefully remove the aluminium seal from wells A1-A12 of row A.
18. Re-seal the whole plate (A1-H12)* with plastic PCR sealer. Centrifuge briefly at 1500 rpm for 30 seconds.
19. Place the plate in PCR machine and run the PCR amplification protocol as mentioned below.

TEMPERATURE	TIME	CYCLES
50°C	2 min	1
95°C	5 min	1
95°C	15 sec	40
60°C	30 sec	
20°C	15 sec	1

NOTES: *Do not store and handle PCR plates in the same area as the samples, DBS controls and DBS calibrators to avoid contamination.*

The part of the plate between B1 and H12 row is sealed twice. This is to ensure the absence of cross-contamination from plasmid calibrators to other wells.

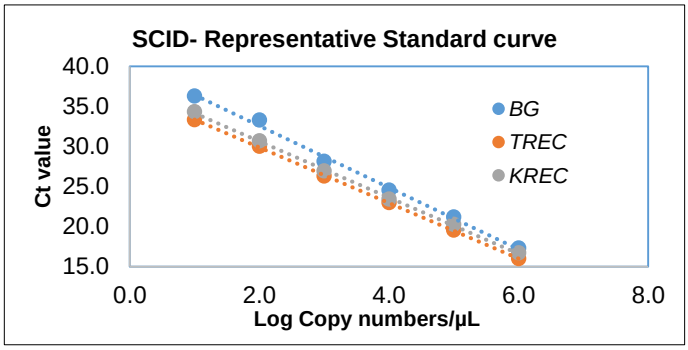
THRESHOLD CYCLE

Programming of the instruments is carried out according to the instrument's user manual. Fluorescence data are plotted against the number of cycles. The threshold cycle (C_t or C_q) serves as a tool for calculation of the starting template amount in each sample. The threshold is adjusted to a value above the baseline, but must be located in the log-linear range of the PCR curve. Before determining the C_t value, ensure that the **baseline is positioned correctly, i.e., between 3 and 15 cycles** and **adjust the threshold, if necessary**. Each laboratory should establish its own threshold.

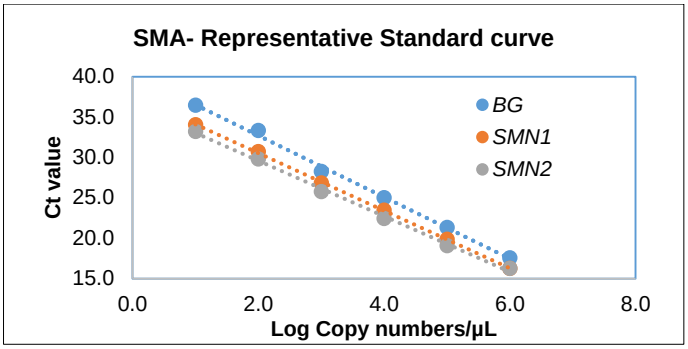
ANALYTICAL PERFORMANCE

Standard Curve

Representative plots of C_t values and concentrations of plasmid calibrators in copies/µl are shown below. This PCR kit is validated using Bio-Rad PCR instrument and all the values are automatically calculated by the Bio-Rad CFX Maestro software. Each laboratory should collect C_t values for plasmid calibrators and build own standard curve for each test plate.



ID	Copies/ µl	C_t value		
		β-globin (BG)	*TREC*	*KREC*
STD 1	10^6	19.6	18,0	19,7
STD 2	10^5	22.9	21,3	23,1
STD 3	10^4	26.3	24,6	26,5
STD 4	10^3	29.7	28,0	30,0
STD 5	10^2	33	31,3	33,3
STD 6	10^1	36.3	34,5	36,7



ID	Copies/ µl	C_t value		
		β-globin (BG)	*SMN1*	*SMN2*
STD 1	10^6	19,7	16,9	15,6
STD 2	10^5	22,9	20,2	18,9
STD 3	10^4	26,3	23,6	22,3
STD 4	10^3	29,7	27,1	25,7
STD 5	10^2	32,9	30,4	29,0
STD 6	10^1	35,9	33,6	32,5

Note: Concentration of DNA for each tested gene in unknown DBS clinical specimens is automatically calculated by the Bio-Rad PCR instrument's CFX Maestro software (using a standard curve built based on the results collected for plasmid calibrators).

Limit of Detection (LOD)/Sensitivity

The Limit of Detection was determined using Bio-Rad PCR instrument's CFX Maestro software in accordance with the CLSI EP17-A2 guidelines. The LOD for each gene is shown below [18].

Genes	Copies/µl
TREC	4
KREC	4.9
SMN1	1.2
SMN2	2.4

Linearity

Linearity of the SCID-SMA assay was evaluated using Bio-Rad PCR instrument's CFX Maestro software according to CLSI EP6-A using 4 DBS specimens. The results are summarized below [19].

Parameter	*β-globin*	*TREC*	*KREC*	*SMN1*	*SMN2*
Range (copies/µL)	10-9306	15-6559	20-14943	05-8959	11-10767
Slope	-0.99	-1.01	-0.95	-1.08	-0.99
X-intercept	4.97	5.22	5.11	5.12	5.04
R square	0.99	1	0.99	0.99	0.99

QUALITY CONTROL

1. A full set of plasmid calibrators and DBS controls must be run in duplicates in each run.
2. Each laboratory should establish mean values and acceptable ranges for C_t values of plasmid/DBS calibrators and DBS controls to assure proper performance.
3. The results should be reported only if the plasmid calibrator and control values meet the acceptance criteria.

Standard curve:

The standard curve parameters should meet the following criteria for the valid assay. This PCR kit is validated using Bio-Rad PCR instrument and the results are confirmed with Quantstudio™ 5 PCR instrument. Since each and every PCR instrument might differ in their assay values, each laboratory should standardaise the assay parameters with their own PCR instrument in accordance with the IFU.

SCID assay

Parameters	*Acceptance Criteria*
	β-globin/TREC/KREC
Efficiency	85-115%
Slope	-2.99 to -3.65
R^2	>0.9

SMA assay

Parameters	*Acceptance Criteria*
	β-globin/SMN1/SMN2
Efficiency	85-115%
Slope	-3.01 to -3.68
R^2	>0.9

Optional: DBS Calibrators

Calibration curve can be also built using the results collected from DBS calibrators.

The theoretical concentration of DNA that are spiked into the DBS calibrators in plasmid copy numbers per µl and the representative C_t values for the extracted plasmid DNA are presented in the following table

ID	Spiked plasmids, copies/ µl	C_t value				
		BG	*TREC*	*KREC*	*SMN1*	*SMN2*
Cal 1	10^6	23.5	23.3	24.4	21.9	22
Cal 2	10^5	26.8	26.6	27.9	25.2	25.4
Cal 3	10^4	30.2	29.9	31.3	28.5	28.7
Cal 4	10^3	33.5	33.4	34.5	31.7	32.1

DBS Controls:

The DBS controls should meet the following criteria for the valid assay.

SCID plate

Controls	***Acceptance Criteria***		
	β-globin, copies/ µl	*TREC,* copies/ µl	*KREC,* copies/ µl
C1	>100	>30	>30
C2	>100	>30	<15
C3	>100	<15	>30
C4	>100	<15	<15

SMA Plate

Controls	***Acceptance Criteria***		
	β-globin, copies/ µl	*SMN1,* copies/ µl	*SMN2,* copies/ µl
C1	>100	>30	>30
C2	>100	>30	<15
C3	>100	<15	>30
C4	>100	<15	<15

RESULTS INTERPRETATION

The standard curve parameters should meet the following criteria for the valid assay. This PCR kit is validated in Bio-Rad PCR instrument and the results are confirmed with Quantstudio™ 5 PCR instrument. Since each and every PCR instrument might differ in their assay values, each laboratory should standardaise the assay parameters with their own PCR instrument accordance with the IFU.

CUT-OFF VALUES

Each laboratory should establish the **cut-off** values by testing large number of normal newborn samples and available positive samples.

1. For SCID assay, concentrations of *TREC, KREC* and *β-globin* in copies/µl can be determined. The results are interpreted as follows:

- *if the value of β-globin is undetermined or lower than 1000 copies/µl, then the result is invalid*. The sample should be retested.
- If concentrations of *TREC* (in copies/µl) can not be calculated or lower than established cut-off value and *β-globin* values are >1000 copies, the sample is considered as SCID positive in screening.
- If concentrations of *KREC* (in copies/µl) can not be calculated or lower than established cut-off value and *β-globin* values are >1000 copies, the sample is considered as SCID & XLA positive in screening (20, 21).

2. For SMA assay, concentrations of *SMN1, SMN2* and *β-globin* in copies/µl can be determined. The results are interpreted as follows:

- *if the value of β-globin is undetermined or lower than 1000 copies/µl , then the result is invalid.* The sample should be tested again.
- If concentration of *SMN1* in copies/µl can not be calculated, SMN2 values can be calculated and β-globin values are >1000 copies, the sample is considered as SMA screen positive.

- **Note:** The quality of extracted DNA and/or the presence of the amplification reaction inhibitors is verified using the β-globin.
- Retesting of samples are recommended when value below or near to cut off
- SMN2 copies or ct values from RT PCR may be used to calculate the actual gene copies in the genome. The laboratory may establish and validate the SMN2 gene copies and in relation to clinical characteristics of patients with SMA (21,22).

The laboratory should use the SCID and SMA screening algorithm adopted in their country.

CLINICAL SENSITIVITY

The screening performance of SCID-SMA kit was evaluated using clinical DBS samples from normal (n=14), SCID positive (n=4), and SMA positive (n=4) newborns.

SCID - Clinical Sensitivity: 100%; clinical specificity: 100%

		SCID		
		Nomal	SCID positive	Total
Clinical diagnosis	Normal	14	0	14
	SCID	0	04	04
	Total	14	04	18

SMA - Clinical Sensitivity: 100%; clinical specificity: 100%

		SMA		
		Nomal	SMA positive	Total
Clinical diagnosis	Normal	14	0	14
	SMA	0	04	04
	Total	14	04	18

LIMITATION OF THE PROCEDURE

a) The kit is not designed to detect “leaky SCID”: a condition when a person has symptoms similar to typical SCID, but with T cell counts that aren't low enough to qualify as typical SCID.
b) The sample discs should be punched carefully as improperly collected samples can cause aberrant results.
c) This assay is sceening assay. Because no single method leads to the definitive diagnosis, the results of the present method should be interpreted in conjunction with the clinical conditionand other laboratory findings.
d) It is recommended that the assay is performed by qualified and trained laboratory technician.

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SYMBOLS USED

Catalog number


CE-mark

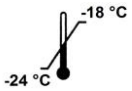
96/192/480
Contains sufficient for <n> tests


Use by YYYY-MM

In vitro diagnostic medical device

Batch code

Temperature limitation for Box 1



Temperature limitation for Box 2


Manufacturer


Consult instructions for use

DBS Control

DBS Calibrator


Potential biohazardous material

gibco
by life technologies

AmnioMAX™ C-100 and AmnioMAX™ II Complete Media

Description

AmnioMAX™ products have been formulated and qualified for the *in vitro* propagation of primary cultures of human amniotic fluid cells and chorionic villus samples for use in prenatal diagnostic testing. AmnioMAX™ products have been optimized to maximize colony attachment, growth rates, pH stability, and to provide prolific metaphasic yield. AmnioMAX™ C-100 Complete Medium consists of an optimized basal medium, AmnioMAX™ C-100 Basal Medium, and supplement, AmnioMAX™ C-100 Supplement, containing an appropriate amount of antibiotics (gentamicin) and growth supplements to eliminate the need for further supplementation. AmnioMAX™ II Complete media is a second-generation formulation to improve cell morphology and provide cleaner cultures in a ready-to-use and convenient format which already containing antibiotics (gentamicin), L-glutamine and FBS. Every manufactured lot of AmnioMAX™ product is tested against rigorous standards to ensure clinical performance.

Product	Catalog No.	Amount	Storage	Shelf Life*
AmnioMAX™ C-100 Complete Medium, kit Kit contains:	12558-011	Kit		
AmnioMAX™ C-100 Basal Medium (1X), liquid	17001-082	90 mL	2°C to 8°C; Protect from light	—
AmnioMAX™ C-100 Supplement	12556-015	15 mL	–20°C to –5°C; Protect from light	—
AmnioMAX™ C-100 Basal Medium (1X), liquid	17001-082 17001-074	90 mL 450 mL	2°C to 8°C; Protect from light	16 months
AmnioMAX™ C-100 Supplement	12556-015 12556-023	15 mL 75 mL	–20°C to –5°C; Protect from light	16 months
AmnioMAX™ II Complete Medium	11269-016	100 mL	–20°C to –5°C; Protect from light	18 months

* Shelf Life duration is determined from Date of Manufacture. Do not use beyond labeled expiration date.

Intended Use

For *in vitro* diagnostic use.

Important Information

Do not use products if:

–Packaging has been compromised
–Product was received completely thawed
–AmnioMAX™ C-100 Basal or AmnioMAX™ II Complete Medium appears cloudy

Safety Information

For every chemical, read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

Prepare Media

AmnioMAX™ II Complete Medium is supplied frozen, ready to use upon thawing. Thaw at 2°C to 8°C, mix by gently swirling to ensure homogeneity. **Do not thaw** at 37°C. This may result in formation of a precipitate and should be avoided.

Note: AmnioMAX™ Media contain Fetal Bovine Serum (FBS); flocculent debris may develop upon thawing and storage.

- Thawed unopened AmnioMAX™ II Complete Medium can be stored in the dark at 2° to 8°C for up to two months within the labeled expiration date.
- Once opened, use AmnioMAX™ products within 7–10 days for maximal growth performance. Repeated warming/cooling and prolonged exposure to light should be avoided.
- **Do not use** beyond labeled expiration date.

Supplement Media

AmnioMAX™ II Complete Medium requires no further supplementation.

AmnioMAX™ C-100 Basal requires supplementation with AmnioMAX™ C-100 Supplement.

1. Aseptically add entire contents (15 mL) of AmnioMAX™ C-100 Supplement to 90 mL AmnioMAX™ C-100 Basal Medium before use.
2. Mix by gently swirling to ensure homogeneity.
3. Store in the dark at 2°C to 8°C until use.

Additional supplementation to AmnioMAX™ products is NOT recommended. **Note:** Addition of Fungizone® may be toxic.

Related Products

Product	Catalog No.
Lab Armor™ Beads	A12543
KaryoMAX® Colcemid™ Solution, liquid (10 µg/mL), in HBSS	15210
KaryoMAX® Colcemid™ Solution, liquid (10 µg/mL), in PBS	15212
KaryoMAX® Giemsa Stain Stock Solution	10092
Gurr Buffer Tablets (pH 6.8)	10582
Phytohemagglutinin (M Form)	10576-015
PB-MAX™ Karyotyping Medium	12557
MarrowMAX™ Bone Marrow Medium	12260

Each clinician/scientist must make an independent judgment on whether this medium is suitable for use in *In Vitro Diagnostic* applications conducted in their laboratory. Life Technologies does not guarantee the successful outcome of any diagnostic testing based solely on the use of GIBCO® medium. Life Technologies contribution to these procedures is simply at the step of providing a culture or handling medium for these procedures.

Explanation of Symbols and Warnings

The symbols present on the product label are explained below:

			
Caution, consult accompanying documents	*In vitro* diagnostic medical device	Sterilized using aseptic processing techniques	Protect from light
			
Use By:	Catalog number	Manufacturer	Batch Code
			
European Community	Consult instructions for use	Temperature Limitation	

Limited Use Label License

No right to resell this product or any of its components is conveyed expressly, by implication, or by estoppel. For information on obtaining additional rights, please contact **outlicensing@lifetech.com** or Out Licensing, Life Technologies, 5791 Van Allen Way, Carlsbad, California 92008.

Limited Product Warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.lifetechnologies.com/termsandconditions**. If you have any questions, please contact Life Technologies at **www.lifetechnologies.com/support**.

For additional technical information such as Safety Data Sheets (SDS), Certificates of Analysis, visit www.lifetechnologies.com/support
For further assistance, email **techsupport@lifetech.com**

life technologies™

AmnioMAX™-C100 and AmnioMAX™-II Complete Medium

Catalog Numbers 12558-011, 17001-082, 12556-015, 17001-074, 17001-094, 17001-095, 12556-023, 12556-096, 11269-016, 11269-097, A5557001

Pub. No. MAN0018473 Rev. C.0

WARNING! Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from **thermofisher.com/support**.

Intended use

AmnioMAX™ products have been formulated and qualified for the in vitro propagation of primary cultures of human amniotic fluid cells and chorionic villus samples for use in prenatal diagnostic testing.

Cytogenetic products are for professional use. They are used in medical laboratories by personnel who have received specialized education and training with regard to procedures utilizing *In Vitro* Diagnostic (IVD) products. IVD products of this type are not intended as sole determinant in a diagnostic situation. Test results are interpreted by a healthcare professional as part of the clinical management of a patient.

Principle and explanation of procedure

Amniotic fluid provides a source of fetal material used in prenatal diagnostic testing. Amniotic fluid samples can be cultured as a source of metaphase cells for chromosome analysis and to provide additional material for biochemical and DNA-based testing

AmnioMAX™ products have been optimized to maximize colony attachment, growth rates, pH stability, and to provide prolific metaphasic yield.

- AmnioMAX™-C100 Complete Medium Kit contains an optimized basal medium and supplement containing Fetal Bovine Serum (FBS), gentamicin, and L-glutamine to maximize cell attachment and growth. This optimized medium also has an enhanced buffering system that provides greater pH stability during culture manipulations. AmnioMAX™-C100 Basal Medium requires supplementation with AmnioMAX™-C100 Supplement.
- AmnioMAX™-II Complete Medium is a second-generation formulation to improve cell morphology and provide cleaner cultures in a ready-to-use and convenient format which already contains antibiotics (gentamcin), L-glutamine, and FBS. AmnioMAX™-II Complete Medium is a nutritionally complete medium and requires no further supplementation.

UK CA CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Contents and storage

All quality control testing results are reported on lot-specific Certificate of Analysis available on our website: **thermofisher.com**.

Product	Cat. No.	Storage	Shelf life[1]
AmnioMAX™-C100 Complete Medium Kit virtual, for ordering puposes only contains: • AmnioMAX™-C100 Basal Medium (1X) • AmnioMAX™-C100 Supplement	• 12558-011 • 17001-082 • 12556-015	—	—
AmnioMAX™-C100 Basal Medium (1X) • 90 mL • 450 mL • 10 × 450 mL • 20 × 90 mL	• 17001-082 • 17001-074[2] • 17001-094 • 17001-095	Protect from light 2°C to 8°C	16 months
AmnioMAX™-C100 Supplement • 15 mL • 75 mL • 20 × 75 mL	• 12556-015 • 12556-023 • 12556-096	Protect from light –20°C to –5°C	16 months
AmnioMAX™-II Complete Medium • 100 mL • 100 mL (For China only) • 20 × 100 mL	• 11269-016 • A5557001 • 11269-097	Protect from light –20°C to –5°C	18 months

[1] Shelf life is determined from Date of Manufacture. Do not use beyond the labelled expiration date.
[2] Dual manufactured.

Related materials

Unless otherwise indicated, all materials are available through **thermofisher.com**. "MLS" indicates that the material is available from **fisherscientific.com** or another major laboratory supplier.

Item	Source
KaryoMAX™ Colcemid™ Solution, liquid (10 µg/mL), in HBSS	15210040
KaryoMAX™ Colcemid™ Solution, liquid (10 µg/mL), in PBS	15212012
KaryoMAX™ Giemsa Stain Stock Solution	10092013
Gurr Buffer tablets (pH 6.8)	10582013
Nunc™ 15 mL Conical Sterile Polypropylene Centrifuge Tubes	339651
Nunc™ 50 mL Conical Sterile Polypropylene Centrifuge Tubes	339653
Nunc™ EasYDish™ Dishes (35 mm)	150460
Nunc™ Thermanox™ Coverslips (25 mm)	174985
Nunc™ Serological Pipettes (5 mL)	170355
HBSS, calcium, magnesium, no phenol red	14025092
PBS	10010023

Precautions

Do not use the product if packaging, including bottles and vials, have been compromised and/or show evidence of microbial contamination, cloudy appearance, discoloration, drying, cracking, or other signs of deterioration. AmnioMAX™-C100 and AmnioMAX™-II Complete Medium should be received frozen; therefore, a thawed product is an indication of a compromised product.

CAUTION! Human amniotic fluid is biohazardous. Follow standard precautions for handling, storage and disposal.

CAUTION! Do not use for injection or infusion! Please report any serious incidents in relation to the device to the manufacturer and the Competent Authority of the EU Member State in which the user and/or patient is established.

Precautions

Do not use the product if packaging, including bottles and vials, have been compromised and/or show evidence of microbial contamination, cloudy appearance, discoloration, drying, cracking, or other signs of deterioration. PB-MAX™ Karyotyping Medium should be received frozen; therefore, a thawed product is an indication of a compromised product.

CAUTION! Human amniotic fluid is biohazardous. Follow standard precautions for handling, storage and disposal.

CAUTION! Do not use for injection or infusion! Please report any serious incidents in relation to the device to the manufacturer and the Competent Authority of the EU Member State in which the user and/or patient is established.

Procedural guidelines

- All solutions that come into contact with clinical samples must be sterile. Always use proper aseptic techniques and work inside a laminar flow hood. Consult our **Gibco Cell Culture Basics** for aseptic handling.
- Perform all incubations in a humidified 37°C, 5% CO_2 incubator unless otherwise specified.

Guidelines for AmnioMAX™-C100 and AmnioMAX™-II Complete Medium

- AmnioMAX™-C100 and AmnioMAX™-II Complete Medium is supplied frozen, ready to use upon thawing.
- Thaw at 2–8°C, then mix by gently swirling to ensure homogeneity. Do not thaw at 37°C. This may result in formation of a precipitate and should be avoided.
- AmnioMAX™ Media contain Fetal Bovine Serum (FBS); flocculent debris can develop upon thawing and storage.
- Thawed, unopened AmnioMAX™-C100 and AmnioMAX™-II Complete Medium can be stored protected from light at 2–8°C for up to two months within the labeled expiration date.
- Once opened, use AmnioMAX™-C100 and AmnioMAX™-II Complete Medium products within 10 days for maximal growth performance.
- Avoid repeated warming/cooling and prolonged exposure to light.
- Do not use beyond the labeled expiration date.

Before you begin

1. Aseptically add the entire contents (15 mL) of AmnioMAX™-C100 Supplement to 90 mL of AmnioMAX™-C100 Basal Medium.
2. Mix by gently swirling to ensure homogeneity.
3. Store protected from light at 2–8°C until use.

   Additional supplementation to AmnioMAX™ products is not recommended. Addition of Fungizone™ can be toxic.

Prepare amniotic fluid samples for culturing

1. Obtain amniotic fluid in 20-mL syringes or tubes that have been approved for cell culture, then maintain samples at room temperature until they are processed.
2. Gently invert the original container of amniotic fluid sample to resuspend cells.
3. Transfer samples to conical tubes (Cat. No. 339651 or 339653), then centrifuge at 100 × *g* at room temperature.
4. Label 35-mm Nunc™ EasYDish™ Dishes (Cat. No. 150460) on both the top and bottom. Using forceps, place sterile Nunc™ Thermanox™ coverslips (Cat. No. 174985) in the bottom of the dishes.
5. Taking care not to disturb the cell pellets, remove the conical tubes from the centrifuge. In the biosafety cabinet, remove the supernatant from each tube. Leave ~1 mL of fluid above the pellet.
6. Transfer the supernatant to another tube to save for future biochemical testing.
7. Carefully aspirate any remaining supernatant above each pellet, then add 0.5 mL of AmnioMAX™-II Complete Medium per culture to be seeded from the pellet.
8. Tap gently on the side of the tube to resuspend the pellet, then distribute the cell suspension equally to the center of the coverslip.

   Be careful to confine the suspension to the coverslip.
9. Incubate cultures for 24 to 48 hours in a humidified incubator at 37°C and 5% CO_2.

Flood the cultures with AmnioMAX™-II Complete Medium

1. Gently add 2 mL of pre-warmed AmnioMAX™-II Complete Medium to the culture dishes.
2. Incubate the cultures for 2 days in a humidified incubator at 37°C and 5% CO_2.

Feed cultures

1. Under the hood, use a sterile 5-mL serological pipette to remove the medium from the periphery of the dishes so that the cells are not disturbed.
2. Add 2 mL of pre-warmed AmnioMAX™-II Complete Medium, then incubate the cultures in a humidified incubator at 37°C and 5% CO_2 for 3 days.

Harvest cells for metaphase chromosome preparation

1. Starting on the fifth day in culture, periodically check for colony formation and cell growth using an inverted microscope.

   Medium should be changed every 48 to 72 hours until colonies are observed.

2. When colony formation is apparent, add 50 µL of 10 mg/mL KaryoMAX™ Colcemid™ Solution (Cat. No. 15210040 or Cat. No. 15212012) to each 2-mL coverslip culture, then gently rotate the dish to mix.

3. Incubate for 20 minutes in a humidified incubator at 37°C and 5% CO_2.

4. Remove the dishes from the incubator, then gently add ~1 mL of hypotonic solution (HBSS Cat. No. 14025092 or PBS Cat. No. 10010023) around the inner edge of dish.

5. Let the dish stand for 10–12 minutes. Aspirate the hypotonic solution/medium mix from around the edge of the dish.

6. Add 2 mL of hypotonic solution, then let the dish stand for 12 minutes.

7. Gently add ~1 mL of fresh 6:1 methanol/glacial acetic acid fixative, then let the dish stand for 12 minutes. Aspirate the fixative solution from around the edge of the dish.

8. Repeat step 7 with fresh 3:1 fixative two or three times for 10 minutes each time.

   Note: Do not remove the final fixative.

9. Lift the coverslip out of the dish with fine forceps, then place the edge of the coverslip on a paper towel to drain excess fixative.

10. Place a 35-mm dish on top of wet paper towels (to create a humid environment for the drying process), then lean the corner of the coverslip (cell side up) against the cover. Allow to dry for 2–3 minutes.

11. Move the 35-mm dish cover to a 60°C slide warmer, then lean the coverslip (cell side up) against the cover. Allow to dry for ~10 minutes.

12. Gently label the back of each coverslip with a Gram stain pen, then leave the coverslips cell side up on the 60°C slide warmer for at least 4 hours, but not more than 24 hours.

13. Stain the coverslips using standard recipes and times for staining slides.

14. After the coverslips are stained and dry, analyze the metaphase chromosomes, then interpret the results.

Stain with Giemsa Stain

Banding of chromosome with enzymes and stains is essential to identifying normal and abnormal chromosome structures.

1. Prepare six Coplin jars according to the following table:

Jar number	Contents
1	0.125% trypsin/0.9% NaCl mixture
2	0.9% NaCl for rinsing
3	0.9% NaCl for rinsing
4	Gurr Giemsa stain (R66) mixed with Gurr 6.8 buffer and acetone
5	Gurr 6.8 buffer for rinsing
6	Gurr 6.8 buffer for rinsing

2. Place a slide for a prescribed amount of time in the jar containing the trypsin/NaCl mixture (Jar 1).

   This time can be as short as 10 seconds or as long as 2 minutes, depending on the activity level of the trypsin being used.

3. After the trypsin time has elapsed, remove the slide, then rinse by sequential dipping into the 0.9% NaCl rinsing jars (Jars 2 and 3).

4. Place the slide in the staining jar (Jar 4) containing the Gurr stain and buffer for 5 minutes.

   This time can vary depending on the strength of the stain used.

5. Remove the slide from the jar, then rinse by sequential dipping into the two Gurr buffer rinsing jars (Jars 5 and 6).

6. Remove the slide from the last rinse to air dry, then coverslip the slide with Cytoseal™ 60.

   It is allowed to dry in the oven (50°C) after which it is ready for metaphase scanning under the microscope.

Quality assurance/control

Every lot of AmnioMAX™-II Complete Medium and AmnioMAX™-C100 Complete Medium is performance tested by a certified US reference cytogenetics laboratory to ensure consistently superior performance. Pooled primary human amniotic fluid samples are cultured for six days in AmnioMAX™-II Complete Medium or AmnioMAX™-C100 Complete Medium before measuring the total number of colonies and total number of mitotic colonies. In addition, each lot is tested for sterility, pH, and osmolality.

Label symbols

The symbols present on the IFU and labels that are not globally recognized as per ISO 15223 are explained in the following table.

Read SDS	READ SAFETY DATA SHEET Consult Safety Data Sheet for risks associated with product.
UK CA	INDICATES CONFORMITY WITH UNITED KINGDOM REQUIREMENTS
UKRP	AUTHORISED REPRESENTATIVE IN THE UNITED KINGDOM

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale at www.thermofisher.com/us/en/home/global/terms-and-conditions.html. If you have any questions, please contact Life Technologies at www.thermofisher.com/support.

UKRP Life Technologies Ltd | 7 Kingsland Grange | Woolston, Warrington WA1 4SR | United Kingdom

EC REP Life Technologies Europe B.V. Kwartsweg 2, 2665 NN Bleiswijk The Netherlands

Life Technologies Corporation | 3175 Staley Road | Grand Island, New York 14072 USA

Revision history: Pub. No. MAN0018473

Revision	Date	Description
C.0	29 July 2022	Catalog numbers were added and minor corrections were made to the product information sheet.
B.0	4 March 2020	• Updated EC Rep address to The Netherlands • Added manufacturing location into Contents and storage table
A.0	15 March 2019	Initial release.

The customer is responsible for validation of assays and compliance with regulatory requirements that pertain to their procedures and uses of the instrument.

The information in this guide is subject to change without notice.

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Media and Reagents for Cytogenetics

- **Optimized and prequalified for cytogenetics**
- **Produce clear, reproducible results that are easy to analyze and interpret**

Clearly Superior Morphology

Figure 1. *Chromosome spread from normal bone marrow cells cultured in GIBCO® MarrowMAX™ Bone Marrow Medium for 24 hours.*

Every day, you make critical decisions based on what you see through a microscope. GIBCO® products for cytogenetics give everything you need to have confidence in your conclusions.

You'll get clear, reproducible results that are simple to analyze and interpret with the most trusted cell culture media and reagents for cytogentics: MarrowMAX™, AmnioMAX™, PB-MAX™, and KaryoMAX® products.

Optimized for Cytogenetics, Certified for Consistency

MarrowMAX™, AmnioMAX™, PB-MAX™, and KaryoMAX® products are specifically formulated and application tested by an independent laboratory to deliver clear, reproducible results in standard clinical cytogenetic protocols involving the analysis of bone marrow cells, amniotic fluid cells, chorionic villus samples, and peripheral blood lymphocytes.

Our production process and strict quality control ensure that every lot of MarrowMAX™, AmnioMAX™, PB-MAX™, and KaryoMAX® product will support healthy cell morphology and rapid culture growth.

Peace of Mind with Each Product

For over 40 years, GIBCO® products for cell culture have set the global standard for quality and reliability.

Our products for cytogenetics are manufactured in compliance with the FDA's Quality System Regulation (cGMP) and the current requirements of ISO 9001 at our Grand Island, New York facility.

You can rest assured that we maintain rigorous control of all production stages, from raw materials to finished product. Our controlled manufacturing process and decades of experience ensure lot-to-lot consistency and reproducible performance.

We Know What Matters

At Invitrogen, we understand the high level of service and support required in cytogenetics laboratories, so we strive to help you in every way possible.

Have questions? Need data? Call Cell Culture Technical Services, 1-800-955-6288 or go to www.invitrogen.com.

GIBCO®
cell culture

GIBCO® Media and Reagents for Cytogenetics

Description	Catalog No.	Size
MarrowMAX™ Bone Marrow Medium (contains Gentamicin) *Recommended storage condition:* -5˚C to -20˚C	12260-014	100 ml
AmnioMAX™-II Complete Medium (contains Gentamicin) *Recommended storage condition:* -5˚C to -20˚C	11269-016	100 ml
AmnioMAX™-C100 Complete Medium (system) The system contains both the medium (90 ml) and the supplement (15 ml). (supplement contains Gentamicin)	12558-011	set
AmnioMAX™-C100 Basal Medium, liquid *Recommended storage condition:* 2˚C to 8˚C	17001-082 17001-074	90 ml 450 ml
AmnioMAX™-C100 Supplement, liquid *Recommended storage condition:* -5˚C to -20˚C (supplement contains Gentamicin)	12556-015 12556-023	15 ml 75 ml
PB-MAX™ Karyotyping Medium Contains fetal bovine serum, phytohemagglutinin, and antibiotics. *Recommended storage condition:* -5˚C to -20˚C (supplement contains Gentamicin)	12557-013 12557-021	100 ml 500 ml
KaryoMAX® Colcemid™ Solution, liquid (10 µg/ml), in HBSS	15210-040	10 ml
KaryoMAX® Colcemid™ Solution, liquid (10 µg/ml), in PBS Prepared in Hanks' Balanced Salt Solution or Phosphate-Buffered Saline. *Recommended storage condition:* 2˚C to 8˚C	15212-012	10 ml
KaryoMAX® Giemsa Stain Stock Solution	10092-013	100 ml
Antibiotic-Antimycotic (100X), liquid* Prepared with 10,000 units of penicillin (base), 10,000 µg of streptomycin (base), and 25 µg of amphotericin B/ml utilizing penicillin G (sodium salt), streptomycin sulfate, and amphotericin B as Funigizone™ Antimycotic in 0.85% saline. *Spectrum:* bacteria, fungi, and yeasts *Recommended storage condition:* -5˚C to -20˚C	15240-096 15240-062	20 ml 100 ml
Collagenase, lyophilized* Prepared from *Cl. histolyticum*. *E.C. Number:* 3.4.2.4.3 *Activity:* 125 to 200 units/mg weight. One unit will liberate 1 µmol amino acid from collagen, expressed as L-leucine, in 18 h at 37˚C *Recommended storage condition:* 2˚C to 8˚C	17018-029	500 mg
Fetal Bovine Serum, Certified (US) Performance, mycoplasma, virus, bacteriophage, and endotoxin tested. *Endotoxin level:* ≤10 EU/ml *Hemoglobin level:* ≤15 mg/dl *Recommended storage condition:* -5˚C to -20˚C	16000-036 16000-044	100 ml 500 ml
Fetal Bovine Serum, Qualified (US) Performance, mycoplasma, virus, and endotoxin tested. *Recommended storage condition:* -5˚C to -20˚C	26140-087 26140-079 26140-095	100 ml 500 ml 1,000 ml
Fetal Bovine Serum, Qualified, Heat-Inactivated (US) Performance, mycoplasma, endotoxin, and virus tested. *Recommended storage condition:* -5˚C to -20˚C	16140-063 16140-071	100 ml 500 ml

Description	Catalog No.	Size
Fungizone™ Antimycotic, liquid*	15290-018	20 ml
Prepared with 250 µg of amphotericin B and 205 µg of sodium deoxycholate/ml as a stabilizer in distilled water. *Spectrum:* fungi and yeasts *Recommended concentration:* 0.25 to 2.5 µg/ml *Recommended storage condition:* 2˚C to 8˚C		
Gentamicin Reagent Solution (10 mg/ml), liquid*	15710-064	10 ml
Prepared with 10 mg/ml gentamicin sulfate in distilled water. *Spectrum:* gram-positive and gram-negative bacteria *Recommended concentration:* 0.5 to 50 µg/ml *Recommended storage condition:* 15˚C to 30˚C	15710-072	10 × 10 ml
Gurr Buffer Tablets (pH 6.8)*	10582-013	50 × 1 L
Optimized to prepare a liter of buffer at pH 6.8 for dilution of Giemsa stain.		
Hanks' Balanced Salt Solution (HBSS) (1X), liquid	14170-120	100 ml
Contains no calcium chloride, magnesium chloride, or magnesium sulfate. *Recommended storage condition:* 15˚C to 30˚C	14170-112	500 ml
	14170-161	10 × 500 ml
Hanks' Balanced Salt Solution (HBSS) (1X), liquid	14175-095	500 ml
Contains no calcium chloride, magnesium chloride, magnesium sulfate, or phenol red. *Recommended storage condition:* 15˚C to 30˚C	14175-079	1,000 ml
	14175-103	10 × 500 ml
L-Glutamine-200 mM (100X), liquid	25030-149	20 ml
Supplied at 29.2 mg/ml in 0.85% NaCl. *pH:* 4.7 to 5.6 *Recommended storage condition:* -5˚C to -20˚C	25030-081	100 ml
Minimal Essential Medium (MEM) Alpha Medium (1X), liquid	12571-063	500 ml
Supplemented MEM Alpha Contains L-glutamine, ribonucleosides, and deoxyribonucleosides. *Recommended storage condition:* 2˚C to 8˚C	12571-071	10 × 500 ml
	12571-048	1,000 ml
Pancreatin 4X USP (10X), liquid*	002-0036DG•	100 ml
Contains 25.0 g of pancreatin and 8.5 g of NaCl/L. *Dilution:* Aseptically prepare 1X solution in balanced salt solution without calcium magnesium to achieve a concentration of 2.5 g/L pancreatin *Recommended storage condition:* -5˚C to -20˚C •Available as custom formulation.		
Penicillin-Streptomycin, liquid*	15140-148	20 ml
Prepared with 10,000 units of penicillin (base) and 10,000 µg of streptomycin (base)/ml utilizing penicillin G (sodium salt) and streptomycin sulfate in 0.85% saline. *Spectrum:* gram-positive and gram-negative bacteria *Recommended storage condition:* -5˚C to -20˚C	15140-122	100 ml
Penicillin-Streptomycin, liquid*	15070-063	100 ml
Prepared with 5,000 units of penicillin (base) and 5,000 µg of streptomycin (base)/ml utilizing penicillin G (sodium salt) and streptomycin sulfate in 0.85% saline. *Spectrum:* gram-positive and gram-negative bacteria *Recommended storage condition:* -5˚C to -20˚C		
Penicillin-Streptomycin-Glutamine (100X), liquid*	10378-016	100 ml
Contains 10,000 units of penicillin (base), 10,000 µg streptomycin (base), and 29.2 mg of L-Glutamine/ml in 0.85% saline, in a 10 mM citrate buffer to maintain penicillin potency. Utilizes penicillin G (sodium salt) and streptomycin sulfate. *Spectrum:* gram-positive and gram-negative bacteria *Recommended storage condition:* -5˚C to -20˚C		

Description	Catalog No.	Size
Phytohemagglutinin (M Form) (PHA), lyophilized*	10576-015	10 ml
Lyophilized extract of the red kidney bean *Phaseolus vulgaris*. Capable of inducing blastogenesis *in vitro* in various mammalian mononuclear cells. PHA is a crude sodium chloride extract of beans that have been homogenized in a saline solution at 2˚C to 8˚C. *Concentration:* Because of the undefined nature of this extract, we are not able to assign weight/volume values on the active material in our final product. *End use:* 1 to 2 ml of rehydrated PHA per 100 ml of culture medium. **Note:** PHA may appear cloudy at refrigerated temperatures (2˚C to 8˚C). This turbidity should clear as the rehydrated solution is brought to room temperature. *Recommended storage condition before rehydration:* 2˚C to 8˚C *Recommended storage condition after rehydration:* -5˚C to -20˚C		
Potassium Chloride Solution, 0.075 M	10575-090	4 × 100 ml
A premixed 0.075 M hypotonic solution. *Recommended storage condition:* 2˚C to 8˚C		
RPMI 1640 (1X), liquid	11875-101	100 ml
Contains L-glutamine and phenol red.	11875-127	20 × 100 ml
Recommended storage condition: 2˚C to 8˚C	11875-093	500 ml
	11875-119	10 × 500 ml
	11875-085	1,000 ml
	11875-135	6 × 1,000 ml
RPMI 1640 (1X), liquid (Folate-Free)*	27016-021	500 ml
Contains L-glutamine, but no folic acid. *Recommended storage condition:* 2˚C to 8˚C		
Trypsin, 2.5% (10X), liquid*	15090-046	100 ml
Porcine parvovirus and mycoplasma tested. Contains 25 g/L of trypsin (1:250) and 8.5 g/L of NaCl, but no phenol red. *Source:* porcine *Recommended storage condition:* -5˚C to -20˚C		
Water, distilled*	15230-162	500 ml
Membrane filtered, cell culture tested, and endotoxin screened.	15230-147	1,000 ml
	15230-196	20 × 100 ml (case)
	15230-204	10 × 500 ml (case)

Invitrogen™ life technologies

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GIBCO® cell culture

Certificate of Analysis

QC Code: GIBCO

AmnioMAX(TM) - II Complete
+ Gentamicin Sulfate
+ L-Glutamine

Lot Number:	2277179
Item Number:	11269
Expiration Date:	2023-10
Storage Temp:	-5 to -20C
Storage Instructions:	Store in the dark

For In Vitro Diagnostic Use

TEST	TEST ID	SPECIFICATION	RESULT	UNITS
Colony Number Actual	CYTOGE0006	>=8.000	8.000	
Cytogenetic Performance Assay	CYTOGE0004	Acceptable	Acceptable	
HCV Antibody Screening	VIRUS0115	Non Reactive	Non Reactive	
Hepatitis B Surface Antigen Screening	VIRUS0113	Non Reactive	Non Reactive	
HIV 1 & 2 Antibody Screening	VIRUS0114	Non Reactive	Non Reactive	
Mitotic Colonies	CYTOGE0009	>=25.0	52.0	%
Osmolality	OSMO0002	280 - 335	310	mOsm/kg
pH	PH0003	7.2 - 7.6	7.3	
Sterility Testing	STERI0006	Negative	Negative	

Read SDS.

GIBCO BRL cell culture liquid products are prepared by an aseptic process for which each step has been validated to ensure that all products meet the industry standard sterility assurance level of 10^-3; i.e. product that demonstrates a contamination level of no more than 1 of 1,000 units during the manufacturing process. The highest level of sterility assurance (equal to or greater than 10^-6) cannot be achieved without terminal sterilization which is harmful to the performance of cell culture products.

Notice: Effective 01/09/2014, the specification for this product has changed per CO#: 35341. Test specifications Colony Number Control and Mitotic Colonies Control have been removed and test specification Mitotic Colonies Actual has been replaced with Mitotic Colonies. If you have questions regarding this change, please contact Thermo Fisher Scientific Technical Support at 1-800-955-6288 in North America or Techsupport@lifetech.com globally.

Quality Systems Department Date: 25-May-2022

References

- CYTOGE0006: Colony number actual: This number represents the mean value for colony number for the actual lot. This is a parameter assessed by Cytogenetic Performance Assay.
- CYTOGE0004: Testing is performed by a leading clinical cytogenetic reference laboratory. Each lot is tested utilizing a six (6) day in situ growth assay using pooled primary amniotic fluid cells. Each test is analyzed using 2 parameters: 1.) Mitotic Colonies and 2.) Colony Number. Detailed information is available as noted below.
- VIRUS0115: FDA Licensed / Approved blood screening tests.
- VIRUS0113: FDA Licensed / Approved blood screening tests.
- VIRUS0114: FDA Licensed / Approved blood screening tests.

- CYTOGE0009: Mitotic colonies actual: This number represents the number of mitotic colonies expressed as a percentage of total Colony Number. This parameter is assessed by Cytogentetic Performance Assay.
- OSMO0002: Thermo Fisher Scientific Specifications.
- PH0003: Thermo Fisher Scientific Specifications.
- STERI0006: Current edition of USP.

МиниМед®
поставщик лабораторий

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www.minimed.ru e-mail: info@minimed.ru

исх. №190 от 26.06.2018г.

Информационное письмо.

В соответствии с действующим Постановлением Правительства РФ от 01.12.2009г. №982 «Об утверждении единого перечня продукции, подлежащей обязательной сертификации, и единого перечня продукции, подтверждение соответствия которой осуществляется в форме принятия декларации о соответствии», Набор реагентов "Масло иммерсионное" по ТУ 9398 -011-29508133-2009, изготовитель ООО «МиниМед», ОКП 939816 «Наборы реагентов для клинической лабораторной диагностики», ОКПД2 21.20.23.110 «Реагенты диагностические», обязательной сертификации и декларированию не подлежит.

Начальник ОТК **Грузинцев С.А.**

Certificate of Analysis

QC Code: GIBCO

KaryoMAX(R) Giemsa Stain
Improved R66 Solution 'Gurr'

Lot Number:	2448624
Item Number:	10092
Expiration Date:	2023-11
Storage Temp:	15 to 30C
Storage Instructions:	Protect from light

For In Vitro Diagnostic Use

TEST	TEST ID	SPECIFICATION	RESULT	UNITS
Chrom 10 Banding Resolution Act Std. Dev	PERBLD0007	Check and Record	5.738	
Chrom 10 Banding Resolution Actual	PERBLD0006	Check and Record	32.880	
Chrom 10 Banding Resolution Cont Std Dev	PERBLD0009	Check and Record	4.336	
Chrom 10 Banding Resolution Control	PERBLD0008	Check and Record	33.240	
Peripheral Blood Performance Testing	PERBLD0001	Acceptable	Acceptable	
- Testing is performed on the raw material.				

Read SDS.

Storage and handling precautions: Limit product exposure to air. Cap tightly.

Recommended Dilution Instructions: Peripheral Blood Chromosomes: 3 mL Giemsa Stain, 48.5 mL Gurr Buffer (pH 6.8)

Repack of Lot 76863785 / 8800725

Quality Systems Department Date: 10-May-2022

References

- PERBLD0007: The chormosome 10 banding resolution represents the standard deviation for the mean value for the actual lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0006: The chormosome 10 banding resolution represents the mean value for the actual lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0009: The chromosome 10 banding resolution represents the standard deviation for the mean value for the control lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0008: The chromosome 10 banding resolution represents the mean value for the control lot. This is a parameter assessed by the Peripheral Blood Performance Test, see reference.
- PERBLD0001: The Karyomax range of media and reagents are evaluated for performance using a test system comparable to the protocols routinely used in cytogenetic laboratories. Test samples of the reagent or media are run in parallel against a previously qualified control lot in a system pertinent to the normal usage in a cytogenetic laboratory. The resulting preparations are analyzed for statistical defference in mitotic index and colony quantity and quality or banding resolution where relevant.

GIBCO® media and culture supplements for cytogenetic analysis

- → Optimized and prequalified for cytogenetics
- → Provide high mitotic index
- → Deliver excellent chromosomal morphology
- → Produce clear, reproducible results that are easy to analyze and interpret

Every day, you make critical decisions based on what you see through a microscope. When your cytogenetics analysis is supported by GIBCO® media and culture supplements, you can be confident in the conclusions you reach.

You'll get clear, reproducible results that are simple to analyze and interpret when you use the most trusted cell culture media and reagents for cytogenetics: MarrowMAX™, AminoMAX™, and PB-MAX™ products.

Superior performance

- → High mitotic index and superior chromosomal morphology (Figure 1)
- → Outperforms commercially available giant cell tumor conditioned medium (GCT-CM) (Figure 2)
- → Consistent lot-to-lot performance (Figure 3)

Convenience

- → Complete, ready-to-use medium
- → Fully supplemented with serum, gentamicin, and L-glutamine
- → Store either frozen or refrigerated

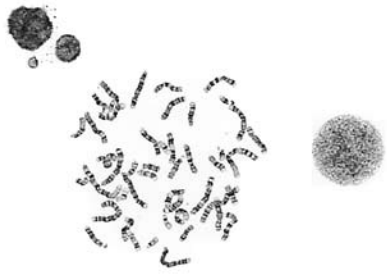

Figure 1—Chromosome spread from bone marrow cells. Cells were cultured in MarrowMAX™ Medium for 24 hours, and G-banding analysis was performed.

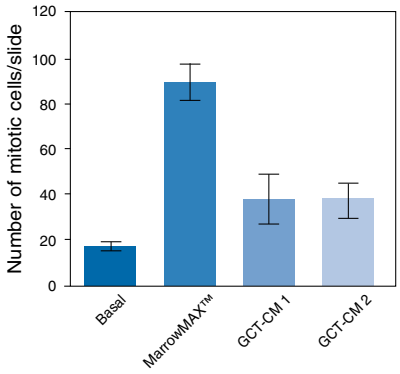

Figure 2—Performance of MarrowMAX™ Medium. Cells were cultured in: basal medium without conditioned medium; MarrowMAX™ Medium; Supplier 1 Medium (GCT-CM 1); and Supplier 2 medium (GCT-CM 2) (both Supplier 1 and Supplier 2 media contain GCT-conditioned medium). Mitotic cells were assayed 24 hours after plating.

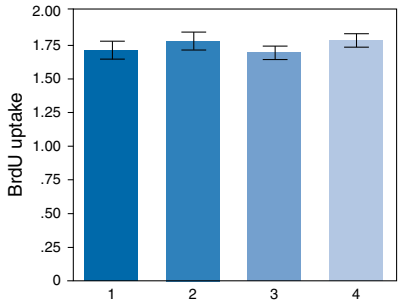

Figure 3—Consistency of MarrowMAX™ Medium. Normal bone marrow mononuclear cells were seeded at 1 x 10^5 cell/ml in 4 different lots of MarrowMAX™ Medium. BrdU uptake was measured by absorbance at 405 nm. Results are mean ± SEM for n = 10.

invitrogen™

Cytogenetic and Diagnostic Testing

Peace of mind with each product

- → Manufactured in compliance with the FDA's Quality System regulation (cGMP) and the current requirements of ISO 9001
- → Application-tested by an independent, certified cytogenetics laboratory to deliver clear, reproducible results in standard clinical cytogenetic protocols
- → Extended shelf life of 18 months when stored unopened at –20°C and 60 days stored at 4°C

We know what matters

At Invitrogen, we understand the high level of service and support required in cytogenetics laboratories, so we strive to help you in every way possible. Have questions? Need data? Contact Invitrogen's cytogenetics specialists at 1 800 955 6288 or visit www.invitrogen.com/cytogenetics.

Ordering Information

Product	Size	Cat. no.
MarrowMAX™ Bone Marrow Medium * (contains gentamicin)	100 ml	12260-014
AmmioMAX™–II Complete Medium (contains gentamicin)	100 ml	11269-016
AmnioMAX™–C100 Complete Medium (system) The system contains both the basal medium (90 ml) and the supplement (15 ml) (supplement contains gentamicin)	1 set	12558-011
AmnioMAX™–C100 Basal Medium, liquid	90 ml 450 ml	17001-082 17001-074
AmnioMAX™–C100 Supplement, liquid (contains gentamicin)	15 ml 75 ml	12556-015 12556-023
PB-MAX™ Karyotyping Medium (contains gentamicin)	100 ml 500 ml	12557-013 12557-021
KaryoMAX™ Colcemid® Solution, liquid (10 mg/ml), in HBSS	10 ml	15210-040
KaryoMAX™ Colcemid® Solution, liquid (10 mg/ml), in PBS	10 ml	15212-012
KaryoMAX™ Giemsa Stain Stock Solution	100 ml	10092-013
Fungizone® Antimycotic, liquid	20 ml	15290-018
Phytohemagglutinin (M Form) (PHA), lyophilized †	10 ml	10576-015

* This product is subject to Limited Use Label License No. 31. These products are for in vitro use and are not intended for human or animal therapeutic use. Uses other than the labeled intended use may be a violation of federal law. † The noted products are for laboratory use only and not for diagnostic use. The safety and efficacy of these products in diagnosis or other clinical uses has not been established. Colcemid® is a registered trademark of CIBA-GEIGY Corporation.

Visit us at www.invitrogen.com/cytogenetics to learn about related reagents for cytogenetic cell culture.

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GIBCO®
invitrogen cell culture

www.invitrogen.com

MarrowMAX™ Bone Marrow Medium

P R O D U C T N E W S

- ***Optimized for cytogenetic analysis***
- ***High mitotic index***
- ***Excellent chromosomal morphology***
- ***Consistent performance***

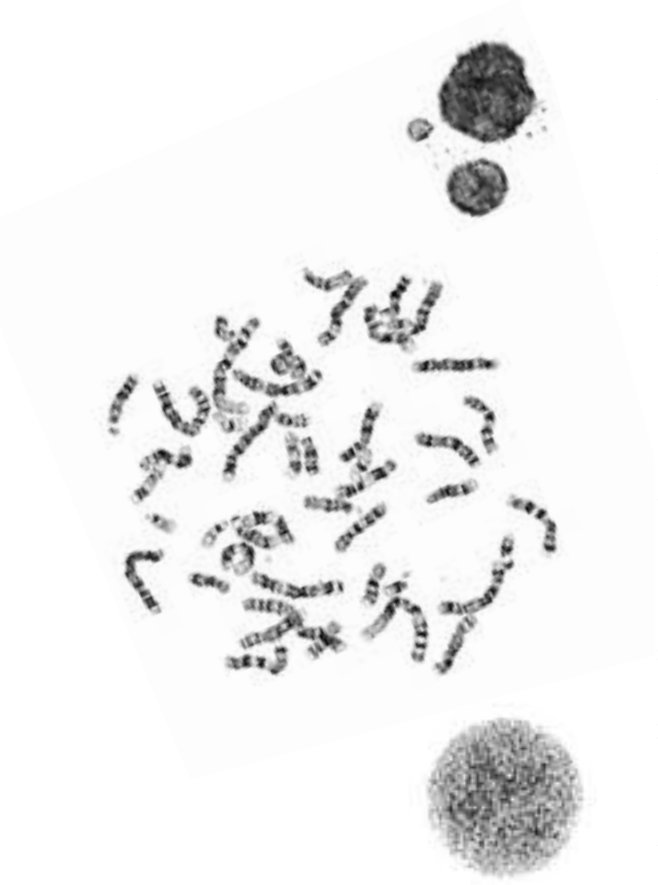

Figure 1. Chromosome spread from normal bone marrow cells. Cells were cultured in MarrowMAX™ Medium for 24 h.

Analysis of human tumors and hematopoietic cells for diagnosis of malignancies is a rapidly growing area of clinical cytogenetics. For the short-term culture of bone marrow, peripheral blood, and hematopoietic cells required for these analyses, many labs use Giant Cell Tumor (GCT) conditioned media containing a variety of hematopoietic growth factors to supplement serum-containing cultures. However, it is difficult to achieve consistent high levels of analyzable cells with commercially available or homemade formulations supplemented with GCT.

GIBCO™ MarrowMAX™ Bone Marrow Medium is a fully supplemented medium developed specifically to support bone marrow and peripheral blood cell culture for *in vitro* cytogenetic analysis of hematological disease.

MarrowMAX™ Bone Marrow Medium contains a novel human stromal cell conditioned medium. This conditioned medium is composed of a unique blend of hematopoietic growth factors for optimal cell growth. The medium is manufactured under strict controls ensuring consistent performance and superior chromosomal morphology *(figure 1)*. Using MarrowMAX™ Medium results in cultures with a high mitotic index and an increased number of analyzable cells.

Superior Performance

- Outperforms commercially available media containing GCT *(figure 2, reverse)*.
- Higher mitotic index and superior chromosomal morphology.
- Consistent lot-to-lot performance *(figure 3, reverse)*.

Convenience

- Complete, ready-to-use medium.
- Fully supplemented with serum, antibiotics, and L-glutamine.
- Store either frozen or refrigerated.

Quality Assurance

- Manufactured in compliance with the FDA's Quality System Regulation (cGMP) and the current requirements of ISO 9001.
- Application tested by an independent certified cytogenetics laboratory using human bone marrow cells.
- Extended shelf life of 18 months when stored unopened at –20˚C and 60 days stored at 4˚C.

GIBCO™
cell culture

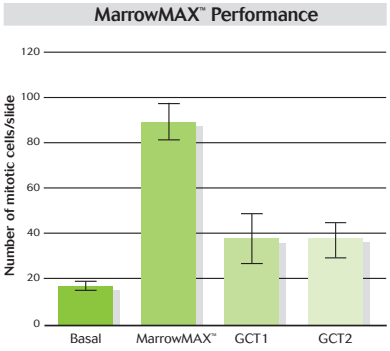

Figure 2. Comparison of media for stimulation of mitotic cells. Cells were cultured in basal medium without conditioned medium (Basal), MarrowMAX™ Medium which contains stromal cell-conditioned medium (MarrowMAX™), Supplier 1 Medium containing GCT-conditioned medium (GCT 1), and Supplier 2 Medium containing GCT-conditioned medium (GCT 2). Mitotic cells were assayed 24 h after plating. Results are mean ± SEM for N = 10 with up to 30 donors.

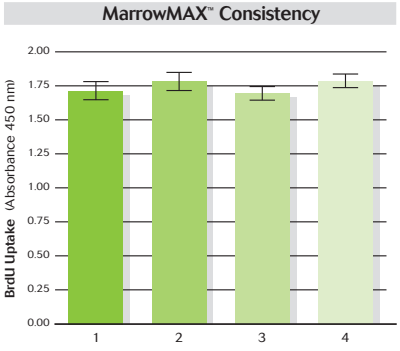

Figure 3. MarrowMAX™ Medium consistency. Normal bone marrow mononuclear cells were seeded at 1 × 10^5 cells/ml in 4 different lots of MarrowMAX™ Medium. Results are mean ± SEM for N = 10.

Ordering Information

Description	Cat. No.	Size
MarrowMAX™ Bone Marrow Medium** (contains gentamicin)	12260-014	100 ml
Related Products		
Complete Media		
AmnioMAX™-II Complete Medium (contains gentamicin)	11269-016	100 ml
AmnioMAX™-C100 Complete Medium (system) — The system contains both the medium (90 ml) and the supplement (15 ml) (supplement contains gentamicin)	12558-011	1 Set
AmnioMAX™-C100 Basal Medium, liquid	17001-082 17001-074	90 ml 450 ml
AmnioMAX™-C100 Supplement, liquid (supplement contains gentamicin)	12556-015 12556-023	15 ml 75 ml
PB-MAX™ Karyotyping Medium (supplement contains gentamicin)	12557-013 12557-021	100 ml 500 ml
Reagents		
KaryoMAX® Colcemid® Solution, liquid (10 µg/ml), in HBSS	15210-040	10 ml
KaryoMAX® Colcemid® Solution, liquid (10 µg/ml), in PBS	15212-012	10 ml
KaryoMAX® Giemsa Stain Stock Solution	10092-013	100 ml
Gurr Buffer Tablets (pH 6.8)*	10582-013	50 × 1 L
Phytohemagglutinin (M Form) (PHA), lyophilized*	10576-015	10 ml

See Chapter 3 of the 2003 GIBCO™ Catalog for more related products.

Invitrogen™ life technologies

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GIBCO™ cell culture

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