PB-MAX™ Karyotyping Medium

Catalog Numbers 12557-013, 12557-021, 12557-097, 12557-098

Pub. No. MAN0018538 Rev. D.0

Intended use

PB-MAX™ Karyotyping Medium has been formulated and qualified for the in vitro propagation of primary cultures of peripheral blood lymphocytes for cytogenetic studies and *In Vitro* Diagnostic (IVD) procedures.

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 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

- The blood cell karyotyping method was developed to provide information about chromosome abnormalities. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48–72 hours, a mitotic inhibitor is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation, and staining, chromosomes can be microscopically observed and evaluated for abnormalities.
- PB-MAX™ Karyotyping Medium has been optimized to maximize colony attachment, growth rates, and to provide prolific metaphasic yield. PB-MAX™ Karyotyping Medium is an optimized RPMI 1640 medium and supplement containing Fetal Bovine Serum (FBS), L-glutamine, and phytohemagglutinin (PHA). PB-MAX™ Karyotyping Medium is based on formulations referenced in the Association for Cytogenetic Technologists Laboratory Manual for the culture of peripheral blood lymphocytes for cytogenetic analysis. PB-MAX™ Karyotyping Medium is a nutritionally complete medium and requires no further supplementation.
- This product is sterile filtered.

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]
PB-MAX™ Karyotyping Medium: • 100 mL • 500 mL • 20 × 100 mL • 10 × 500 mL	• 12557-013 • 12557-021 • 12557-097 • 12557-098	Protect from light –20°C to –5°C	12 months

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

CE IVD

For In Vitro Diagnostic Use.

ThermoFisher SCIENTIFIC

Related products

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
Phytohemagglutinin (M form)	10576015
KaryoMAX™ Potassium Chloride Solution	10575090
Trypsin 2.5%, no phenol red	150900
Trypsin-EDTA (0.5%) 10X	15400054
Nunc™ 15mL Conical Sterile Polypropylene Centrifuge Tubes	339651
Nunc™ Serological Pipettes (5 mL)	170355

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 blood 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

- 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 PB-MAX™ Karyotyping Medium

- PB-MAX™ Karyotyping 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.
- PB-MAX™ Media contain Fetal Bovine Serum (FBS); flocculent debris can develop upon thawing and storage.
- PB-MAX™ Karyotyping Medium can be thawed and aseptically transferred into smaller aliquots for convenience. Aliquots can be frozen and thawed at time of use. Multiple freeze-thaw cycles should be avoided.
- Once opened, use PB-MAX™ Karyotyping Medium products within 14 days for maximal growth performance.
- Avoid repeated warming/cooling and prolonged exposure to light.
- Do not use beyond the labeled expiration date.

Culture peripheral blood lymphocytes for chromosome analysis (Phytohemagglutination assay)

1. Inoculate ~0.5 mL of heparinized whole blood into a glass or plastic tube with 10 mL of PB-MAX™ Karyotyping Medium.
2. Incubate the culture for 72 hours in a humidified incubator at 37°C and 5% CO_2.
3. Add 0.5 µg/mL of KaryoMAX™ Colcemid™ Solution (Cat. No. 15212012 or Cat. No. 15210040) to each culture tube.
4. Incubate the culture for 15–30 minutes in a humidified incubator at 37°C and 5% CO_2.
5. Transfer the culture to a centrifuge tube, then centrifuge at 500 × *g* for 5 minutes.
6. Remove the supernatant, then resuspend the cells in 5–10 mL of 0.075 M KCl (KaryoMAX™ Cat. No. 10575090).
7. Incubate for 10–12 minutes in a humidified incubator at 37°C and 5% CO_2.
8. Centrifuge at 500 × *g* for 5 minutes.
9. Remove the supernatant, agitate the cellular sediment, then add drop-by-drop 5–10 mL of fresh, ice-cold 3:1 methanol/glacial acetic acid fixative. Incubate at 4°C for 10 minutes.
10. Incubate for 10–12 minutes in a humidified incubator at 37°C and 5% CO_2.
11. Centrifuge at 500 × *g* for 5 minutes.
12. Resuspend the cell pellet in 0.5–1 mL of fresh fixative, then drop onto a clean slide to air dry.

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 PB-MAX™ Karyotyping Medium is performance tested by a certified US reference cytogenetics laboratory to ensure consistently superior performance. Peripheral blood lymphocytes from a normal adult donor are cultured for 72 hours in PB-MAX™ Karyotyping Medium before measuring the mitotic index and chromosome banding resolution. In addition, each lot is tested for pH and osmolality, and must pass a sterility test prior to lot release.

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.
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**.

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

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Revision history: Pub. No. MAN0018538

Revision	Date	Description
D.0	26 January 2023	Updated manufacturing address to Paisley. Removed UKCA symbol. Minor edits
C.0	29 July 2022	Catalog numbers were added and minor corrections were made to the product information sheet.
B.0	4 March 2020	The EC Rep address was updated.
A.0	15 March 2019	Initial release.