## Product Name

Name: PBType-1 Peripheral Blood Karyotyping Medium

Cat. No.: C3601-0005, C3601-0100, C3601-0150

Size: 5 mL, 100 mL, 5 mL*30

## Product Description

PBType-1 Peripheral Blood Karyotyping Medium is intended for short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. PBType-1 Peripheral Blood Karyotyping Medium is based on RPMI 1640 basal medium supplemented with L-Glutamine, fetal bovine serum (FBS), Dulbecco's Phosphate Buffered Saline, without Calcium, Magnesium, antibiotic (gentamicin), and phytohemagglutinin-M (PHA-M).

PBType-1 Peripheral Blood Karyotyping Medium is supplied as a frozen medium, which is ready for use after thawing.

## Note

- It is OK to use if a visible precipitate is observed in the medium.
- Use of PBType-1 Peripheral Blood Karyotyping Medium does not guarantee the successful outcome of any chromosome/karyotyping analysis testing.
- Do not use PBType-1 Peripheral Blood Karyotyping Medium beyond the expiration date indicated on the product label.

## Storage and Stability

The product should be kept at **-20°C**.
The product is **light-sensitive** and therefore should not be left in the light.
Shelf life: 24 months from date of manufacture.

## Procedure

Thaw PBType-1 Peripheral Blood Karyotyping Medium at refrigerator temperatures (2 - 8°C) or by swirling the bottle in a 37°C water bath. Mix gently during thawing.

Note that the medium already contains L-Glutamine, antibiotic, and PHA-M.

## Culture of Peripheral Blood Lymphocytes for Chromosome Analysis

The Blood cell karyotyping method has been developed to provide information about chromosomal abnormalities. Lymphocytes do not normally undergo subsequent cell divisions. In the presence of a mitogen, lymphocytes are stimulated to enter into mitosis by DNA replication. After 48 - 72 hours, a mitotic inhibitor (Colcemid Solution) 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.

1. Inoculate approximately 0.5 mL of heparinized whole blood into a glass or plastic tube with 10 mL of PBType-1 Peripheral Blood Karyotyping Medium.
2. Incubate the culture for 72 hours.
3. Add 0.1 - 0.2 mL of Colcemid Solution to each culture tube. Incubate the culture for an additional 15 - 30 minutes (The volume or concentration can be changed according to the experimental requirements).
4. Transfer the culture to a centrifuge tube and spin at 500 x *g* for 5 minutes.
5. Remove the supernatant and re-suspend the cells in 5-10 mL hypotonic 0.075 M KCl solution to lyse red blood cells. Incubate at 37ºC for 10 – 12 minutes.
6. Spin at 500 x *g* for 5 minutes.
7. Remove the supernatant, agitate the cellular sediment, and add drop-by-drop 5 - 10 mL of fresh, ice-cold fixative made up of 1-part acetic acid to 3 parts methanol. Leave at 4ºC for 10 minutes.
8. Repeat steps 6 and 7.
9. Spin at 500 x *g* for 5 minutes.
10. Resuspend the cell pellet in a small volume (0.5 - 1 mL) of fresh fixative, drop onto a clean slide, and allow to air-dry.
11. At this stage, the preparation can be stained with Orcein or Giemsa. (Giemsa banding is the most common method to obtain the staining and the slides needed to treat with trypsin-EDTA 10X solution for a few seconds).

**Quality Control**

PBType-1 Peripheral Blood Karyotyping Medium is tested for sterility, pH, osmolality, and endotoxin concentration. In addition, each batch is tested for karyotyping in a leading clinical cytogenetics laboratory for its performance.

**Quality Assurance**

- Manufactured under ISO 13485 QMS.
- Manufactured under controlled environments and processes in accordance with:
  1. ISO 13408 – Aseptic Processing of Health Care Products
  2. ISO 14644 – Cleanrooms and associated controlled environments.

**Precaution and Disclaimer**

For research use only, not for clinical diagnosis, and treatment.