

# Technical Data Sheet

## LymphoFroxx (peripheral blood) Complete Medium

for chromosome analysis

Order number: 2471

LymphoFroxx (peripheral blood) Complete Medium is intended for use in short-term cultivation of peripheral blood lymphocytes for chromosome evaluation. The medium is based on a basal medium, supplemented with L-Glutamine, fetal bovine serum, antibiotics (penicillin/streptomycin), and phytohemagglutinin-M (PHA-M). Blood cell karyotyping of lymphocytes is an important tool in modern human cytogenetics to detect chromosomal abnormalities. Lymphocytes usually do not undergo subsequent cell divisions. In the presence of a mitogen such as PHA-M, lymphocytes are stimulated to enter mitosis. After 48 – 72 hours, a mitotic inhibitor (e.g. Colcemid) 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.

### Instruction for use

**Please note:** LymphoFroxx Complete Medium already contains L-Glutamine, antibiotics, and PHA-M. It is supplied as frozen medium, which is ready for use after thawing. Further supplementation is not required nor recommended.

1. Thaw LymphoFroxx Complete Medium and make aliquots of 10 ml (sterile tubes).
2. Continue warming up the precalculated amount of LymphoFroxx Complete Medium (in tubes of 10 ml) until room temperature is reached.
3. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml LymphoFroxx Medium.
4. Incubate the culture (37°C, 5 % CO<sub>2</sub>) in an incubator for 72 hours.
5. Add 0.1 – 0.2 ml of Colcemid Solution to each culture tube (at a final concentration of 0.1 µg/ml). Incubate the culture for additional 15 – 30 minutes.
6. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.
7. Remove the supernatant and resuspend the cells in 5 – 10 ml of hypotonic 0.075 M KCl (prewarmed to 37°C). Incubate at 37°C for 10 – 12 minutes.
8. Spin at 500 g for 5 minutes.
9. 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 4°C for 10 minutes.
10. Repeat steps 8 and 9.



11. Spin at 500 g for 5 minutes.
12. 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.
13. At this stage, the preparation can be stained with Orecin or Giemsa. For Giemsa staining, the most widely used method, you can use one of the common staining protocols or the protocol established in your laboratory.

### Storage and handling

Store LymphoFroxx Complete Medium at -20°C protected from light. Thaw LymphoFroxx Complete Medium at 2 – 8°C or put medium bottle in a 37°C water bath and swirl gently to homogenize. **Do not use if a visible precipitate is observed in the medium.** Once opened, store at 2 - 8°C and use within 10 days.

### Related products

2473	Colcemid solution (10 µg/ml in DPBS) for chromosome analysis
2474	Phytohemagglutinin M (PHA-M) solution for chromosome analysis
2364	AmnioFroxx (type A) Complete Medium for amniotic fluid cells and chorion villi samples
2466	AmnioFroxx (type B) Complete Medium for amniotic fluid cells and chorion villi samples
1229	Sodium hydrogen carbonate for cell biology
1500	Water sterile for cell biology
1429	D-PBS (1X) w/o Ca and Mg (pH 7.4) for cell biology
6085	Hanks' Balanced Salt Solution w/o Ca and Mg, w/o Phenol red, with NaHCO <sub>3</sub>
4673	Earle's Balanced Salt Solution w/o Ca and Mg, with Phenol red and NaHCO <sub>3</sub>
2472	Trypsin (0.5 %) - EDTA (0.2 %) 10X solution in DPBS w/o Ca and Mg, w/o Phenol red
1444	Trypsin (0.05 %) - EDTA (0.02 %) solution in HBSS w/o Ca and Mg, with Phenol red
1501	Trypsin (0.25 %) - EDTA (0.53 mM) solution in HBSS, w/o Ca and Mg, with Phenol red
4266	Trypsin inhibitor (1 mg/ml) for cell biology

Not intended for therapeutic use. Each laboratory is obliged to perform representative tests according to the valid legal regulations and in its own environment to ensure that it is suitable for this purpose before the medium can be used in routine diagnostics.

Use of LymphoFroxx Complete Medium does not guarantee the successful outcome of any prenatal diagnostic testing.

Do not use LymphoFroxx Complete Medium beyond the expiration date indicated on the product label.

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