CAUTION

Handle in accordance with established bio-safety practices.

Designated Purpose
Lymphogrow serves the cultivation and growth of human lymphocytes and is to be used exclusively for *in vitro* diagnostic purposes on samples taken from humans.

Composition
Basal media, Phytohaemagglutinin (PHA) high quality, pre tested FBS. Buffered with HCO^3.
With antibiotics (Pen/Strep) and L-Glutamine.

Shelf Life and Storage
Lymphogrow is stable for 15 months after production when stored at ≤-18 °C. Due to quality and sterility Lymphogrow should be used within a maximum of 2 days after opening and storing at +2 °C to +8 °C.

Thawing
Lymphogrow Medium can be thawed at +2 °C to +8 °C overnight or in a +37 °C water bath. Mix medium thoroughly by swirling prior to use. In case of pH variation (pink or yellow) open cap slightly (about ¼ turn) and incubate bottle in a 5% CO^2 incubator until normal coloration (phenol red). Lymphogrow does not contain components sensitive to pH changes of +/- 2. Warm medium at the proper pH is best for the initialization of cultures.

Protocol for Use
The protocol below provides a guide for peripheral blood lymphocyte cell culture using Lymphogrow. The medium is bottled under aseptic conditions. The maintenance of sterility is absolutely necessary for the use in *in vitro* diagnostics and must be strictly adhered to by the user. This high quality medium can naturally be used within established procedures. It is up to the user to adopt either parts of or all of the optimized protocol described below.

Cell Culture Protocol
- Thaw Lymphogrow and make aliquots of 5 ml (sterile tubes)
- Thaw the pre-calculated amount of Lymphogrow medium (in tubes) until room temperature is reached
- Transfer 0.5 ml of heparinized whole blood into a tube containing 5 ml Lymphogrow
- Mix and incubate at +37 °C, 5% CO^2 in an incubator for 48 to72 hours
- 1 – 2 hours before the end of the incubation period, add 0.1 ml of Colcemid (at a final concentration of 0.1 µg/ml)

Harvesting Protocol
- Centrifuge (5 minutes at 500 x g)
- Discard the supernatant, (leave a few drops at the bottom)
- Add 5 – 10 ml of 0.075 M KCl heated to +37 °C (mix while dispensing)
- Leave for 10 minutes at room temperature
- Centrifuge (5 minutes at 500 x g)
- Add 5 – 8 ml of fixative (freshly prepared 3 parts Methanol: 1 part Acetic acid)
- Repeat the last two steps twice
- Re-suspend the cell pellet in a small volume of fresh fixative
- For analysis of the karyotype follow your usual laboratory procedure

Important Remarks
- For *in vitro* diagnostic use only (IVD)
- CAUTION: Not for human or animal therapeutic use. Uses other than the intended use may be a violation of local law.
- Each laboratory must carry out their own testing procedures on new media according to national legislation prior to releasing them to the lab for routine *in vitro* applications.
- 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.
- Cytogen GmbH does not guarantee the successful outcome of any diagnostic testing based solely on the use of Cytogen brand medium.

CE marked
With Lymphogrow, Cytogen offers a CE marked medium for IVD which fulfils the requirements of the directive 98/79/EC defined by the European Commission.

MANUFACTURER

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