

Attention: The patient samples are biological material and therefore safety precautions are necessary.

Purpose of Use

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 NaHCO₃.
With antibiotics (Pen/Strep) and L-Glutamine.

Shelf Life and Storage

Lymphogrow Medium is durable at a storage ≤-18 °C for 15 months from the date of manufacture. For quality and sterility reasons, the use of Lymphogrow medium is recommended after opening at a storage of + 2 °C to + 8 °C of a maximum of 7 days. Repeated thawing and freezing should be avoided. Lymphogrow Medium not exceed the expiry date indicated on the label.

Thawing

Thaw Lymphogrow medium at + 2 °C to + 8 °C overnight. Thawing in a water bath at 37 °C is not recommended. Mix well before using Lymphogrow medium. The normal pH value is 7,2 as indicated by the phenol red indicator. In the case of a pH deviation (yellow or pink), the pH value is obtained by incubating the slightly open bottle (approx. ¼ rotation of the lid) in a 5% CO₂ incubator equilibrated until the medium has reached the normal color red. Lymphogrow medium contains no components whose quality is affected by pH fluctuations of +/-2. Heated medium at 37 °C and correct pH-value ensures an optimal start of the culture.

Protocol

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 cell culture bottle containing 5 ml Lymphogrow
- Mix and incubate at +37 °C, 5% CO₂ in an incubator for 48 to 72 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 observation

- Calciumoxalat crystals may occasionally form, but have not yet shown any negative effects on cell growth.
- Thawing in a water bath at 37 °C should be avoided since precipitates can form.

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

Cytogen Produkte für Medizin + Forschung GmbH
Nordwalder Str. 120
48268 Greven
Germany

Tel. +49 2571 560180
Fax +49 2571 9219118
E-Mail: info@cytogen.net
Web: www.cytogen.net

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