

Intended use

Lymphogrow is used for cultivating human lymphocytes from peripheral blood. It is intended for human genetic *in vitro* diagnostics.

Composition

Basal medium, phytohaemagglutinin (PHA), pretested FBS, phenol red, buffered by NaHCO₃; contains penicillin-streptomycin and L-glutamine.

Shelf life and storage

Unopened Lymphogrow medium can be stored for 18 months from the date of manufacture if stored at ≤-18°C. After opening, store the bottle at +2°C to +8°C and use for a maximum of 7 days. Avoid repeated thawing and freezing.

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 method described below is a guide for using Lymphogrow in the cultivation of lymphocytes from peripheral blood. 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.

Recommendation for cultivation

- Thaw Lymphogrow and aliquot, e.g., 5 ml each (sterile tubes).
- Thaw the amount of Lymphogrow you are going to use at room temperature.
- Add 0.5 ml of heparinised whole blood to a cell culture flask containing approx. 5 ml of medium.
- Mix and incubate for 48 to 72 hours at +37 °C, 5% CO₂ in the incubator.
- 1–2 hours before the end of incubation, add 0.1 ml colcemid (final concentration 0.1 µg/ml) and mix gently

Recommendation for cell harvesting

- Preheat 0.075 M potassium chloride at 37 °C
- Centrifuge for 5 minutes at 500x g
- Discard supernatant (do not remove completely)
- Add 5–10 ml of warmed 0.075 M potassium chloride solution to the cells and mix thoroughly.
- Incubate at room temperature for 10 minutes.
- Centrifuge for 5 minutes at 500x g
- Prepare the fixative solution fresh on ice (3 parts methanol to 1 part acetic acid).
- Discard supernatant (do not remove completely)
- Add 5–8 ml of fixative solution
- Repeat the last two steps (incubation and centrifugation) 2 x
- Resuspend cell pellet in a small volume of fixative solution

- Proceed according to the appropriate protocol for karyogram preparation.

Important notes

- Occasionally, calcium oxalate crystals can form, but, to date, they have not shown any negative effects on cell growth.
- Thawing in a water bath at 37°C. should be avoided, as precipitates can form.

Warnings and precautions

- Maintaining the sterility of the product is necessary for its use, and this must be strictly adhered to by the user.
- Not suitable for therapeutic use with humans or animals.
- May only be used by trained, specialist personnel.
- Do not use bottles whose packaging has been damaged.
- Do not use Lymphogrow Medium beyond its expiry date, which is stated on the label.
- The patient samples constitute biological material, and, therefore, safety precautions must be taken in accordance with local regulations for working with potentially infectious material.
- Cytogen GmbH does not guarantee the success of diagnostic testing solely through the use of Cytogen products.
- Report serious incidents that have occurred in connection with this product to the manufacturer and the competent authorities.

CE marking

With Lymphogrow, Cytogen offers a CE-marked medium that meets the regulatory requirements for *in vitro* diagnostic medical devices set by the European Commission.

Explanation of symbols

	Observe instructions for use		Manufacturer
	<i>In vitro</i> diagnostic medical device		Date of manufacture
	Article number		Sterilised using aseptic methods
	Production lot number, batch		Do not use if the packaging is damaged
	Use by		Keep away from sunlight
	Temperature limit		

Manufacturer

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