Lymphochrome Media

Product Information

BioWhittaker™ Lymphochrome

- 02-015X5-Box: 10 x 5 ml
- 02-015E: 100 ml
- 02-015F: 500 ml

CAUTION

Handle in accordance with established bio-safety practices.
Product Feature Summary

Intended Use

**Lymphochrome** is intended to grow peripheral blood lymphocytes, which are used for use in vitro diagnostic procedures. The medium does contain the mitogen phytohemagglutinin (PHA). The mitogen-induced cell growth results in cells that are suitable for karyotyping, Fluorescence-In-Situ-Hybridisation (FISH) and other cytogenetic procedures. The product has been rigorously quality control tested by a leading European clinical cytogenetic diagnostic reference laboratory for this application.

Background

Peripheral blood lymphocytes are widely used in cytogenetic labs for cytogenetic studies. Lymphocyte cells are mature cells in which mitosis can be stimulated using mitogens like PHA. Those stimulated cells are suitable for chromosome analysis\(^1\). Cytogenetic labs use often complete media that consist of a base medium (DMEM, CMRL 1066 or RPMI1640) supplemented with foetal bovine serum (FBS), PHA, L-glutamine and antibiotics\(^2\).

**BioWhittaker™ Lymphochrome** is ready to use formulation based on a serum free medium that contains PHA, L-Glutamine and Gentamycin as antibiotic. The serum free formulation shows less lot-to-lot variability and save the time usually required for testing serum batches.

Product Description

**Lymphochrome** (02-015) is supplied frozen, ‘ready-to-use’ and does not require any supplementation. The medium does contain Phytohaemagglutinin P (PHA-P), L-Glutamine and the antibiotic Gentamycin offering additional convenience to the end-user.

The medium is buffered with Sodium Bicarbonate as well as HEPES and Phenol Red is present as a pH indicator. The complete formulation reduces the chance of technical error and culture contamination.

Lymphochrome is offered in 100 ml and 5 ml configurations. The 5 ml tubes are available in packs of 10 tubes.

Storage Conditions and Shelf-life

**BioWhittaker™ Lymphochrome**

**Storage:** Store at < -18°C and in the dark.

**Shelf life:** Two years from day of manufacture when stored at recommended storage conditions.

**Limitation:** Do not use more than two years after manufacturing date.

Instruction for Use

Media Preparation

**BioWhittaker™ Lymphochrome** - should be thawed at 4°C to 8°C. Excessive temperature will degrade heat labile nutrients. Prior to use warm up to room temperature and gently swirl to mix completely.

Thawed medium can be stored 2°C to 8°C for up to two weeks. Repeated warming / cooling and prolonged exposure to light should be avoided.

Medium can be as well aseptically transferred into smaller aliquots. Those aliquots can be frozen and thawed at time of use. Multiple freeze thaw cycles should be avoided.

Antibiotics

**Lymphochrome** contains Gentamycin, which is less inhibitive to growth compared to Penicillin and Streptomycin.

Performance Testing

**Lymphochrome Medium** is tested for sterility by EP and analysed for pH, and endotoxin content. In addition to these standard specifications, each manufactured lot is tested for performance by an independent European Cytogenetics laboratory and compared to a reference standard. A Certificate of Analysis (CoA) is available upon request.

Precautions
Please contact directly the Sales & Marketing Department at Lonza for any concerns relating to the product or ask your local distributor to do it on your behalf.

Do not use product if:

- Packaging appears compromised.
- Product appears cloudy or a visible precipitate is observed.

If product is received thawed or partially thawed, freeze immediately at < -18°C and contact Lonza.

FOR USE IN IN VITRO DIAGNOSTIC PROCEDURES REQUIRING THE CULTIVATION AND GROWTH OF PERIPHERAL BLOOD LYMPHOCYTES ONLY.

Additional supplementation to Lymphochrome Medium is not recommended. Adding components or diluting the medium may result in negative effects on cell growth or chromosome integrity.

Limitations

Each laboratory must carry out their own testing procedures on new media prior to releasing them to the lab for routine in vitro applications. Lonza contribution to these procedures is simply to provide a culture or handling medium and therefore Lonza do not guarantee the successful outcome of any testing based only on the use of Lonza medium.

Lonza recommends testing the patient samples in duplicate; using a cell culture medium previously released.

Each manufactured lot of Lymphochrome Medium is thoroughly performance tested on peripheral blood lymphocytes to ensure product performance for in vitro diagnostic use for this application.

Lonza cell culture liquid products are prepared by an aseptic process for which each step has been validated to ensure that all products meet the industry standard sterility assurance level of $10^{-3}$ i.e., product that demonstrates a contamination level of no more than 1 of 1000 units during the manufacturing process. The highest level of sterility assurance (equal to or greater than $10^{-6}$) cannot be achieved without terminal sterilization, which is harmful to the performance of these cell culture products.

Cell Culture Protocols

The protocols below provide a guide for peripheral blood lymphocyte culture using Lymphochrome Medium. This can be used to replace either part of or all of existing optimised protocols peripheral blood lymphocyte cultures at the user’s discretion. The majority of cytogentic laboratories have their own protocols and Lymphochrome Medium can, in most cases, be simply substituted in current cell culture protocol.

The following additional reagents are needed to perform the whole procedure (but not supplied):

- Colcemid® 10 µg/ml
- KCl solution, 0.075 M
- Methanol/Acetic acid 3:1

1) Thaw the appropriate number of Lymphochrome tubes and let them reach room temperature
2) Sterile transfer 0.5 ml of heparinized whole blood into the tube
3) Mix and incubate at 37°C and 5% CO₂ in a incubator for 48 to 72 hours (tubes must lie horizontally)
4) 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)
5) Centrifuge (5 minutes at 500 g)
6) Discard the supernatant almost completely, (leave a few drops at the bottom)
7) Add 5-10 ml of KCl 0.075 M pre-heated to 37°C (mix while dispensing)
8) Leave for 10 minutes at room temperature
9) Centrifuge (5 minutes at 500 g)
10) Add 5-8 ml of fixative, (freshly prepared 3 parts Methanol: 1 part Acetic acid)
11) Repeat steps 9 and 10 twice.
12) Re-suspend the cell pellet in a small volume of fresh fixative
Should it be necessary or more convenient to store the cell pellet, it is recommended to seal the tube carefully and place it in a −20°C freezer.

For the Karyotype analysis follow your own laboratory standard procedure.

REFERENCES

For In Vitro Diagnostic Use.

Not to drink.
No to inject to human or animal.

CAUTION: Not for human or animal therapeutic use. Uses other than the labeled intended use may be a violation of local law.

MANUFACTURER
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The following chart displays the symbols with their definitions.

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