

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

Purpose of Use

Mitogen Free Medium serves the cultivation and growth of human blood cancer or tumor cells and is to be used exclusively for *in vitro* diagnostic purposes on samples taken from humans.

Composition

Basal media, no mitogen whatsoever, pre-tested FBS. Buffered with NaHCO₃. With antibiotics (Pen/Strep).

Shelf Life and Storage

Mitogen Free Medium is durable at a storage ≤-18 °C for 24 months from the date of manufacture. For quality and sterility reasons, the use of Mitogen Free 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. Mitogen Free Medium not exceed the expiry date indicated on the label.

Thawing

Thaw Mitogen Free medium at + 2 °C to + 8 °C overnight. Thawing in a water bath at 37 °C is not recommended. Mix well before using Mitogen Free 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. Mitogen Free 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 cell culture using Mitogen Free Medium. 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 Mitogen Free Medium (use mitogen if necessary) and make aliquots of 5 ml (sterile tubes)
- Thaw the pre-calculated amount of Mitogen Free Medium (in tubes) until room temperature is reached
- Transfer 0.5 ml of heparinized whole blood into a tube containing 5 ml Mitogen Free Medium
- 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 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.

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