Phytohaemagglutinin-M (PHA-M) liquid

CAT N°: L3010

Storage conditions : Store frozen medium at -20°C
After thawing, the PHA-M is stable for at least 1 month at +2/+8°C. The PHA-M may appear cloudy at +2/+8°C, but this turbidity has no effect on the activity of the product.

Shelf life : 36 months

Composition : After thawing, each ml of the solution contains 5 to 10 mg of protein.

Recommended use :
- Respect storage conditions of the product
- Do not use the product after its expiry date
- Store the product in a dry area
- Wear clothes adapted to the manipulation of the product to avoid contamination (e.g. : gloves, mask, hygiene cap, overall…)
- Protect the product from any form of humidity
- Use, in one time, after opening, the entire quantity of product of the container, without making a concentrated solution (to avoid the formation of precipitates). If it is not possible, close the container immediately after sampling the quantity of powder required.
- Supplements can be added prior to sterile filtration of the medium or aseptically introduced to sterile medium (respect the final concentration of the media). The nature of the supplements may affect storage conditions and shelf life of the medium.

The product is intended to be used in vitro, in laboratory only. Do not use it in therapy, human or veterinary applications.

Application:
Phytohaemagglutinin is a lectin extracted from red kidney beans (Phaseolus vulgaris). The protein consists of two molecular species : a leucoagglutinin (PHA-L)and an erythroagglutinin (PHA-E). Each of the proteins contains a family of five isolectins, each being a tetramer held together by noncovalent forces. PHA-M is the mucoprotein form and is a crude extract used for the stimulation of cell proliferation in lymphocyte culture. PHA-M also has a powerful erythroagglutinating property and it was originally used for separating leukocytes from whole blood.

Preparation instructions:
1) Add 2-4 ml of PHA-M per 100 ml of your karyotyping medium.
2) Inoculate approximately 0.5 ml of heparinised whole blood into a glass or plastic tube with 10 ml of medium (or 10^6 viable cells per ml).
3) Incubate the culture for 72 hours.
4) Add 0.1-0.2 ml of Colcemid solution (ref : L0040) to each culture tube.
5) Incubate the culture for 15-30 minutes.
6) Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.
7) Remove the supernatant and re-suspend the cells in 5-10 ml of hypotonic 0.075 M KCl (ref : L0643).
8) Incubate at 37°C for 10-12 minutes.
9) Spin at 500 g for 5 minutes.
10) Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5-10 ml of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol.

11) Leave in 4°C for 10 minutes.
12) Repeat steps 10, 11 and 12.
13) Spin at 500 g for 5 minutes.
14) Re-suspend the cell pellet in a small volume 0.5-1 ml of fresh fixative, drop onto a clean slide and allow to air dry.
15) At this stage, the preparation can be stained with Orecin or Giemsa. Giemsa banding has become the most widely used technique. The most common method to obtain this staining is to treat slides with Trypsin-EDTA 10X (ref : X0930).

Indications of deterioration:
Medium should be free of particulate and flocculent material.
Do not use this medium if it contains precipitate.
Other evidence of deterioration may include colour change or degradation of physical or performance characteristics.