Phytohaemagglutinin-P (PHA-P) liquid

CAT N°: L3020

Storage conditions: Store frozen medium at -20°C. After thawing, the PHA-P is stable for at least 2 weeks at +2/+8°C. The PHA-P may appear cloudy at +2/+8°C, but this turbidity has no effect on the activity of the product. Avoid repeated freezing and thawing cycles.

Shelf life: 12 months

Composition: PHA-P has a molecular weight of 128 kDa and is not blood group specific.

Colour: colourless, clear solution

pH: 7.25 ± 0.25

Osmolality: 285 ± 25 mOsm/kg

Endotoxin: < 1 EU/ml

Sterility tests:
- Bacteria in aerobic and anaerobic conditions
- Fungi and yeasts

Cell Growth test: Not applicable

Other tests: Not applicable

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 product 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 for research or further manufacturing only and not for use as an Active Pharmaceutical Ingredient or food or animal feed.

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-P is the form of PHA before separation of

erythroagglutinin and leucoagglutinin and is a crude extract used for the stimulation of cell proliferation in lymphocyte culture. PHA-P also has a powerful erythroagglutinating property and it was originally used for separating leukocytes from whole blood.

Uses:

1) Add 5 ml of PHA-P per 100 ml of your karyotyping medium.
2) Inoculate approximately 0.5 ml of heparinised whole blood into a glass or plastic tube / flask with approximately 5 ml of medium.
3) Incubate the culture for 72 hours.
4) 1-2 hours before the end of the incubation period add 0.1 ml of Colcemid solution, ref.: L0040 (at a final concentration of 0.1 µg/ml) to each culture tube / flask.
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.

Signs 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.

Remarks: Not applicable