BI
Biological Industries

Cytogenetics

Phytohaemagglutinin M (PHA-M)

For the stimulation of peripheral blood lymphocytes

Cat. No.: 12-009-1H
Store at: -20°C

Instructions for Use

Product Description

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.

PHA-M is a sterile, frozen solution of an aqueous extract from selected red kidney beans.

Precaution and Disclaimer

- For *in vitro* diagnostic use. PHA-M is not intended for therapeutic use.
- Use of Biological Industries PHA-M does not guarantee the successful outcome of any diagnostic testing.
- Do not use PHA-M beyond the expiration date indicated on the product label.

Storage and Stability

The frozen solution should be stored 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.
The turbidity has no effect on the activity of PHA-M.

Instructions for Use

After thawing, each ml will contain 5-10mg of protein.

Procedure

1. Add 2-4ml of **PHA-M (Cat. No. 12-009-1H)** per 100ml Karyotyping Medium (Cat. No. 01-198-1).
2. Inoculate approximately 0.5ml of heparinized whole blood into a glass or plastic tube with 10ml of medium (or 10^6 viable cells per ml).
3. Incubate the culture for 72 hours.
4. Add 0.1-0.2ml of **Colcemid Solution (Cat. No. 12-004-1)** to each culture tube. Incubate the culture for an additional 15-30 minutes.
5. Transfer the culture to a centrifuge tube and spin at 500g for 5 minutes.
6. Remove the supernatant and re-suspend the cells in 5-10ml of hypotonic **0.075M KCl (Cat. No. 12-005-1)**. Incubate at 37ºC for 10-12 minutes.
7. Spin at 500g for 5 minutes.
8. Remove the supernatant, agitate the cellular sediment and add drop-by-drop 5-10ml of fresh, ice-cold fixative made up of 1 part acetic acid to 3 parts methanol. Leave in 4ºC for 10 minutes.
9. Repeat steps 7 and 8.
10. Spin at 500g for 5 minutes.
11. Re-suspend the cell pellet in a small volume 0.5-1ml of fresh fixative, drop onto a clean slide and allow to air dry.
12. 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 (Cat. No. 03-051-5).**

Quality Control

PHA-M is tested for sterility. In addition, the mitotic stimulation is evaluated using primary human peripheral blood lymphocytes.

References

1. Moorhead, P.S., et al, Chromosome Preparations of Leukocytes Cultured from Human Peripheral Blood, Exp. Cell. Res., 20:613-616 (1960)
2. Nowell, P.C., Phytohemagglutinin - An Initiator of Mitosis in Cultures of Normal Human Leukocytes, Cancer Res., 290:462-466 (1960)
3. Barch, M.J. (ed), The Association of Cytogenetic Technologists Laboratory Manual, Second Edition (1991)

Related Products

Product	Cat. No.
Trypsin EDTA, 10X concentrate	03-051-5
Colcemid Solution	12-004-1
0.075M KCl Solution	12-005-1
PB Karyotyping Medium	01-198-1

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