Product Information, Instructions for Use

Phytohemagglutinin M (PHA-M)
Cat. No.: PHA-K (5 ml)

Product Description

Phytohemagglutinin 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.

Product Specifications

Sterility	Tested
Storage	Store at ≤-15°C. After thawing, the PHA-M is stable for at least 1 month at +2°C to +8°C. PHA-M may appear cloudy at +2°C to +8°C. The turbidity has no effect on the activity of PHA-M.
Working concentration	After thawing, each ml will contain 5 – 10 mg of protein.

Instructions for Use: Culture of Peripheral Blood Lymphocytes for Chromosome Analysis

Blood cell karyotyping of lymphocytes is an important tool in modern human cytogenetics to detect chromosomal abnormalities. Lymphocytes usually do not undergo subsequent cell divisions. In the presence of a mitogen (e.g. PHA), lymphocytes are stimulated to enter into mitosis. After 48 – 72 hours, a mitotic inhibitor (e.g. colcemid) is added to the culture to stop mitosis in the metaphase stage. After treatment by hypotonic solution, fixation and staining, chromosomes can be microscopically observed and evaluated for abnormalities.

1. Add 2 – 4 ml of PHA-M per 100 ml of karyotyping medium.
2. Transfer 0.5 ml of heparinized whole blood into a tube containing 10 ml karyotyping medium supplemented with PHA-M (or 10^6 viable cells per ml).
3. Incubate the culture at +37°C, 5 % CO_2 in an incubator for 72 hours.
4. Add 0.1 – 0.2 ml of Colcemid Solution (Cat. No. COL-H) to each culture tube (at a final concentration of 0.1 µg/ml). Incubate the culture for additional 15 – 30 minutes.
5. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.
6. Remove the supernatant and re-suspend the cells in 5 – 10ml of hypotonic 0.075 M KCl, pre-warmed to +37°C. Incubate at +37°C for 10 – 12 minutes.
7. Spin at 500 g for 5 minutes.
8. 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. Leave at +4°C for 10 minutes.
9. Repeat steps 7 and 8.
10. Spin at 500 g for 5 minutes.

11. 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.
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. TRY-1B10).

Related Products

Product	Cat. No.
Colcemid Solution (10 µg/ml) in DPBS	COL-H
Trypsin-EDTA (0.5 %) in DPBS (10x)	TRY-1B10

Precautions and Disclaimer

- For *in vitro* diagnostic use. PHA-M is not intended for therapeutic use.
- Use of 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.

Help needed?

If you have any further questions regarding this product please do not hesitate to contact our cell culture experts:
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