Product Information

Phytohemagglutinin M (PHA-M)
Cat. No. PHA-H (10 ml)

General Information

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 that contains potent cell agglutinating and mitogenic activities and is most often utilized for the stimulation of cell division in lymphocyte cultures.

Application:

- Stimulates mitotic division of lymphocytes in cytogenetic and immunological applications
- Powerful erythroagglutinating properties

Product Specifications

Appearance	Frozen liquid
Storage and shelf life	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.
Shipping conditions	Frozen (Dry Ice)
Working concentration	2 – 4 ml of PHA-M solution per 100 ml culture medium

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 resuspend 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.

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11. Resuspend 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. For Giemsa staining, the most widely used method, you can use one of the common staining protocols or the protocol established in your laboratory.

Precautions and Disclaimer

For *in vitro* diagnostic use. The solution is not intended for therapeutic use.
Each laboratory is obliged to perform representative tests according to the valid legal regulations and in its own environment to ensure that it is suitable for this purpose before the solution can be used in routine diagnostics.
Do not use if a visible precipitate is observed in the solution.
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.

Signs and Symbols

REF	Order number
LOT	Batch Code
	Storage conditions: temperature limit
	Expiration date
STERILE A	Aseptic filling
IVD	*In vitro* diagnostics

Help Needed?

If you have any further questions regarding this product, please do not hesitate to contact our cell culture experts by email (techservice@capricorn-scientific.com) or phone (+49 6424 944640).