Product Information/ Instruction for Use

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

Intended Purpose

Phytohemagglutinin is a mitogen used to stimulate cell proliferation in lymphocyte cell cultures. Lymphocytes do not usually undergo further cell divisions. In the presence of a mitogen (e.g. PHA), lymphocytes are induced to undergo mitosis, which allows karyotyping of lymphocytes to detect chromosomal abnormalities.

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

Phytohemagglutinin is a non-automatic, *in vitro* Diagnostic class A sterile product intended for laboratory use.

Product Specifications

Appearance	Frozen liquid
Storage and shelf life	Store at ≤-15°C. Do not use this product after its expiry date. 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

For lot specific data (Certificate of Analysis), please refer to our website:
https://www.capricorn-scientific.com/en/services/certificate-of-analysis

Instructions for Use

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.

Culturing of Peripheral Blood Lymphocytes for Chromosome Analysis:

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 106 viable cells per ml).
3. Incubate the culture at +37°C, 5 % CO_2 in an incubator for 72 hours.

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Harvesting of Peripheral Blood Lymphocytes for Chromosome Analysis:

1. 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.
2. Transfer the culture to a centrifuge tube and spin at 500 g for 5 minutes.
3. 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.
4. Spin at 500 g for 5 minutes.
5. 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.
6. Repeat steps 7 and 8.
7. Spin at 500 g for 5 minutes.
8. 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.
9. 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 with humans or animals.
- Application only by trained specialist personnel.
- Maintaining the sterility of the product is necessary for its use and must be strictly observed by the user.
- Do not use PHA-H if the packaging is damaged and thus sterility is impaired.
- 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.
- The patient specimens are biological material and therefore safety precautions must be taken according to local regulations for working with potentially infectious material.
- Use of PHA-H does not guarantee the successful outcome of any diagnostic testing.
- Do not use PHA-H beyond the expiration date indicated on the product label.
- Frozen condition must be maintained until first use.
- Report serious incidents that have occurred in connection with this product to the manufacturer and the appropriate authorities.

Signs and Symbols

REF	Catalog number
LOT	Batch Code
	Storage conditions: temperature limit
	Expiration date

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	Aseptic filling
	*In vitro* diagnostics
	Manufacturer
	Protect from sunlight
	Date of manufacture
	CE Marking
	See instruction manual
	Do not use if packaging is damaged

Manufacturer

Capricorn Scientific GmbH
Auf der Lette 13 A
35085 Ebsdorfergrund
Germany

Safe disposal

Waste treatment methods
The generation of waste should be avoided or minimized wherever possible. Empty containers or liners may retain some product residues. This material and its container must be disposed of in according to approved disposal technique. Disposal of this product, its solutions or of any by-products, shall comply with the requirements of all applicable local, regional or national/federal regulations.

Product
Offer surplus and non-recyclable solutions to a licensed disposal company.

Contaminated packaging
Dispose of as unused product.

Environmental precautions
No special environmental precautions required

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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).