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Not for use in diagnostic procedures.



Phytohemagglutinin-M (PHA-M) from *Phaseolus vulgaris*

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Store lyophilizate at +2 to +8°C.

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1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Phytohemagglutinin-M (PHA-M)	<ul style="list-style-type: none"> Lyophilized Filtered through 0.2 µm pore size membrane prior to lyophilization. 	1 bottle, 20 mg

1.2. Storage and Stability

Storage Conditions (Product)

When stored at +2 to +8°C, the lyophilizate is stable through the expiration date printed on the label.

Vial / Bottle	Label	Storage
1	Phytohemagglutinin-M (PHA-M)	Store at +2 to +8°C. ⚠ Keep protected from light.

Storage Conditions (Working Solution)

Store reconstituted solution for 2 weeks at +2 to +8°C or for several months in aliquots at –15 to –25°C.

Reconstitution

Reconstitute PHA-M in autoclaved double-distilled water (final concentration 2 to 10 mg/ml). It can be further diluted with medium or PBS (phosphate buffered saline).

1.3. Additional Equipment and Reagent required

For reconstitution of lyophilizate and dilution

- autoclaved double-distilled water
- Cell culture medium, such as RPMI 1640, 10% fetal calf serum (FCS) (v/v), 2 mM L-glutamine, 1% non-essential amino acids (v/v) or PBS (phosphate buffered saline)

For measuring growth stimulation of peripheral blood lymphocytes (PBLs)

- Peripheral blood lymphocytes (PBL), isolated with lymphocyte separation medium
- [³H]-thymidine
- Glass fiber filter
- α-β scintillation counter

1.4. Application

Phytohemagglutinin-M (PHA-M) is used as a mitogen and stimulates the cell division in lymphocyte cultures.

2. How to Use this Product

2.1. Protocols

Measure growth stimulation of peripheral blood lymphocytes (PBLs)

The following protocol measures growth stimulation via [³H]-thymidine incorporation.

- 1 Plate 900 µl cell suspension, 1×10^4 PBLs/ml, into each well of a microplate.

- 2 Add 100 µl PHA-M to each well in a dilution series (10 dilutions, range 2 to 20 µg/ml).

- 3 Incubate the mixture for 48 hours at +37°C.

- 4 Add 0.5 µCi/well [³H]-thymidine.

- 5 Continue the incubation for an additional 20 hours.

- 6 Collect the cells and wash on a glass fiber filter.

- 7 Measure the remaining cell bound radioactivity in an α-β scintillation counter.

2.2. Parameters

Biological Activity

Optimum activity for the stimulation of proliferation of lymphocytes is at 5 to 10 µg/ml.

Working Concentration

Use 2 to 10 µg/ml PHA-M for the stimulation of human peripheral blood lymphocytes.

3. Additional Information on this Product

3.1. Test Principle

Phytohemagglutinin (PHA), the lectin extract from the red kidney bean (*Phaseolus vulgaris*), contains potent, cell agglutinating and mitogenic activities. PHA contains a family of five isolectins (L_4E_0 , L_3E_1 , L_2E_2 , L_1E_3 , L_0E_4), each being a tetramer held together by noncovalent forces.

The subunits are of two different types, designated leukocyte reactive (L) and erythrocyte reactive (E).

- Type L has high affinity for lymphocyte surface receptors but little for those of erythrocytes and is responsible for the mitogenic properties of the isolectins.
- Type E is responsible for the erythrocyte agglutinating properties.

PHA-P is the protein form and PHA-M is the mucoprotein form of these isolectins.

Preparation

PHA-M is purified from *Phaseolus vulgaris* (red kidney bean) by standard chromatographic techniques. PHA-M is the mucoprotein form of phytohemagglutinin.

4. Supplementary Information

4.1. Conventions

To make information consistent and easier to read, the following text conventions and symbols are used in this document to highlight important information:

Text convention and symbols

 *Information Note: Additional information about the current topic or procedure.*

 **Important Note: Information critical to the success of the current procedure or use of the product.**

   etc. Stages in a process that usually occur in the order listed.

   etc. Steps in a procedure that must be performed in the order listed.

* (Asterisk) The Asterisk denotes a product available from Roche Diagnostics.

4.2. Changes to previous version

Layout changes.

Editorial changes.

4.3. Trademarks

All product names and trademarks are the property of their respective owners.

4.4. License Disclaimer

For patent license limitations for individual products please refer to:

List of biochemical reagent products.

4.5. Regulatory Disclaimer

For life science research only. Not for use in diagnostic procedures.

4.6. Safety Data Sheet

Please follow the instructions in the Safety Data Sheet (SDS).

4.7. Contact and Support

To ask questions, solve problems, suggest enhancements or report new applications, please visit our **Online Technical Support Site.**

To call, write, fax, or email us, visit **sigma-aldrich.com**, and select your home country. Country-specific contact information will be displayed.

