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

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

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Cat. No. 11 249 738 001 5 mg

Store lyophilizate at +2 to +8°C.

1. General Information 3
1.1. Contents 3
1.2. Storage and Stability 3
Storage Conditions (Product) 3
Storage Conditions (Working Solution) 3
Reconstitution 3
1.3. Additional Equipment and Reagent required 3
1.4. Application 3
2. How to Use this Product 4
2.1. Protocols 4
Measure growth stimulation of peripheral blood lymphocytes (PBLs) 4
2.2. Parameters 4
Biological Activity 4
Molecular Weight 4
Working Concentration 4
3. Additional Information on this Product 5
3.1. Test Principle 5
Preparation 5
4. Supplementary Information 6
4.1. Conventions 6
4.2. Changes to previous version 6
4.3. Trademarks 7
4.4. License Disclaimer 7
4.5. Regulatory Disclaimer 7
4.6. Safety Data Sheet 7
4.7. Contact and Support 7

1. General Information

1.1. Contents

Vial / Bottle	Label	Function / Description	Content
1	Phytohemagglutinin-L (PHA-L)	▪ Lyophilized ▪ Filtered through 0.2 µm pore size membrane prior to lyophilization.	1 bottle, 5 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-L (PHA-L)	Store at +2 to +8°C.

Storage Conditions (Working Solution)

Store reconstituted solution in aliquots at −15 to −25°C.
⚠ ***Avoid repeated freezing and thawing.***

Reconstitution

Reconstitute PHA-L in sterile, double-distilled water (final concentration 1 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

- Sterile, 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
- [^{3}H]-thymidine
- Glass fiber filter
- α-β scintillation counter

1.4. Application

Use PHA-L as a high quality tool for functional analysis of T lymphocytes. It is highly purified and tested for high efficiency stimulation of human lymphocytes.

ⓘ *Although PHA-L may be employed for routine lymphocyte culture, the conditions necessary for its successful use are much more stringent then those required for less pure reagents. In particular, the concentration of PHA-L giving maximal lymphocyte stimulation without inhibition or toxicity lies within very narrow limits.*

2. How to Use this Product

2.1. Protocols

Measure growth stimulation of peripheral blood lymphocytes (PBLs)

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

1. Plate 900 µl cell suspension, 1 × 10^4 PBLs/ml, into each well of a microplate.
2. Add 100 µl PHA-L to each well in a a dilution series (10 dilutions, range 1 to 10 µg/ml).
3. Incubate the mixture for 48 hours at +37°C.
4. Add 0.5 µCi/well [^{3}H]-thymidine.
5. Continue the incubation for an additional 20 hours.
6. Collect the cells and wash the cells 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 µg/ml as tested with human peripheral blood lymphocytes.

Molecular Weight

115,000 Da

Working Concentration

Use 1 to 5 µg/ml PHA-L for the stimulation of lymphocytes.
During flow cytometric analysis, use

- for a maximal shift of the cells into the third cell cycle, <5 µg/ml PHA-L,
- for a half-maximal shift, <1 µg/ml PHA-L.

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-L consists only of L-type subunits (isolectin L_4, "leukoagglutinin") and is especially suitable for high efficiency induction of T lymphocytes.

Preparation

Phytohemagglutinin-L (PHA-L) is purified from *Phaseolus vulgaris* (red kidney bean) by standard chromatographic techniques.

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.	
① ② ③ etc.	Stages in a process that usually occur in the order listed.
❶ ❷ ❸ 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.
Purity parameter removed.

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.

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