

# Phytohemagglutinin-M (PHA-M)

From *Phaseolus vulgaris*

Lyophilized, before lyophilization filtered through 0.2 µm pore size membrane

**Cat. No. 11 082 132 001**

20 mg

 **Version 15.0**  
Content version: December 2010

Store at +2 to +8°C  
Store protected from light!

## 1. Product description

### Preparation

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

### Biological activity

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

### Working concentration

For the stimulation of human peripheral blood lymphocytes 2 – 10 µg PHA-M/ml are recommended.

### Contents

Lyophilizate; before lyophilization filtered through 0.2 µm pore size membrane.

### Reconstitution

PHA-M should be reconstituted in sterile redist. water (final concentration: 2 – 10 mg/ml). Further dilution with medium or PBS (phosphate buffered saline).

### Storage and Stability

The lyophilizate is stable at +2 to +8°C through the expiration date printed on the label when stored protected from light. The reconstituted solution is stable for 2 weeks at +2 to +8°C or for several months when stored in aliquots at –15 to –25°C.

## 2. Application

Phytohemagglutinin-M (PHA-M) is used for the stimulation of cell division in lymphocyte cultures.

Phytohemagglutinin (PHA), the lectin extract from the red kidney bean (*Phaseolus vulgaris*), contains potent, cell agglutinating and mitogenic activities (1). PHA contains a family of five isolectins (L<sub>4</sub>E<sub>0</sub>, L<sub>3</sub>E<sub>1</sub>, L<sub>2</sub>E<sub>2</sub>, L<sub>1</sub>E<sub>3</sub>, L<sub>0</sub>E<sub>4</sub>) each being a tetramer held together by noncovalent forces. The subunits are of two different types, designated leucocyte reactive (L) and erythrocyte reactive (E). L has high affinity for lymphocyte surface receptors but little for those of erythrocytes and is responsible for the mitogenic properties of the isolectins. E is responsible for the erythrocyte agglutinating properties. PHA-P is the protein form and PHA-M is the mucoprotein form of these isolectins (1, 2).

PHA is used as a mitogen in lymphocyte cultures (3-6). For the stimulation of cell proliferation in lymphocyte cultures 2 – 10 µg/ml PHA-M lyophilizate are recommended.

\* available from Roche Applied Science

## 3. Procedure for measuring growth stimulation of peripheral blood lymphocytes (PBLs) via [<sup>3</sup>H]-thymidine incorporation.

### Reagents

- Peripheral blood lymphocytes (PBL), isolated with lymphocyte separation medium\*.
- Cell culture medium, e.g., RPMI 1640; 10% fetal calf serum (FCS) (v/v), 2 mM glutamine, 1% nonessential amino acids (v/v)\*.
- PHA-M dissolved in redist. sterile water, 2 – 10 mg/ml.
- [<sup>3</sup>H]-thymidine.

### Procedure

- Seed 1 ml cell suspension/well, 1 × 10<sup>4</sup> PBLs/ml in microtiter plates.
- Add PHA-M to each well in a dilution series (10 dilutions, range 2 – 20 µg/ml).
- Incubate the mixture for 48 h at 37°C.
- Add [<sup>3</sup>H]-thymidine, 0.5 µCi/well.
- Continue the incubation for further 20 h.
- Collect the cells and wash on glass fiber filter.
- Measure remaining cell bound radioactivity in α-β scintillation counter.

### References

- 1 Hammerstrom, S. et al. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 1611-1615.
- 2 Leavitt, R. D. et al (1977) *J. Biol. Chem.* **252**, 2961-2966.
- 3 Dillner-Centerlind, M. L. et al. (1980) *J. Immunol.* **10**, 434-442.
- 4 Scott, M. G. & Nahm, M. H. (1984) *J. Immunol.* **135**, 2445-246G.
- 5 Di Sabato, G. et al. (1987) *Methods Enzymol.* **150**, 1-17.
- 6 Parker, C. W. (1987) *Methods Enzymol.* **150**, 29-83.

### Changes to Previous Version

- Replacement of the wording "sterile"
- Editorial changes.

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