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Purified Phytohaemagglutinin

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PURIFIED PHYTOHAEMAGGLUTININ

Phytohaemagglutinin (PHA), derived from extracts of *Phaseolus vulgaris* seeds, has been used for a number of years, on account of its twin properties of causing erythroagglutination and of stimulating progressive lymphocyte mitosis in cell culture^{3,5,13,18}. Both agglutinating and mitogenic activities appear to be associated with the protein fractions of crude extracts, which are sufficiently alike in their physico-chemical properties to have caused difficulty in attempts to separate them^{1,17,19}. One other factor complicating fractionation procedures has been the slow and imprecise method of assay for the mitogenic activity. Fortunately, relatively crude extracts selected for their ability to yield good mitotic pictures (as in reagent grade Phytohaemagglutinin) have proved entirely satisfactory for use in routine lymphocyte culture for chromosome studies. PHA has proved of interest in the study of the immune response^{2,6,7}, lymphocyte kinetics^{4,9,14,15,20}, and bone marrow dynamics^{8,10,12}, and for these purposes it is desirable that a substance of more closely reproducible qualities and known potency should be used. Improvements in the technique of assaying mitogenic activity¹⁶ have made it possible to quote a value for each batch of Purified Phytohaemagglutinin in terms of a reference standard preparation.

COMPOSITION

Purified Phytohaemagglutinin is a freeze-dried, highly refined protein fraction of selected *Phaseolus* spp. seed extract in which the specific mitogenic activity and the mitogenic/haemagglutinating activity ratio have been increased by a factor of about 100/1 during purification¹¹. Each bottle contains the stated weight and activity of PHA, dried from a small volume of buffered saline without preservative.

PRECAUTIONS

For *in vitro* diagnostic use.

Although Purified Phytohaemagglutinin may be employed for routine lymphocyte culture, the conditions necessary for its successful use are much more stringent than those required for less pure reagents. In particular, the concentration of PHA giving maximal lymphocyte stimulation without inhibition or toxicity lies within very narrow limits. The use of Reagent Grade Phytohaemagglutinin is recommended for all purposes that do not require precise dosage of mitogen with minimal haemagglutinating activity and inactive impurities.

RECONSTITUTION

The reagent may be reconstituted to any desired volume in sterile saline (if buffered, preferably to a pH between 6.5 and 8) or distilled water, using a sterile disposable hypodermic syringe. The cap of the bottle should be sterilized by wiping with ether, the needle should pierce the centre of the rubber plug and be held in a vertical position during reconstitution. If desired, the risk of bacterial contamination of reconstituted Phytohaemagglutinin may be minimized by addition of a few drops of chloroform to the solution: alternatively, antibiotics or any of the usual preservatives may be added if the proposed use of the reagent permits.

LIFE AND STORAGE

The dried material will retain full potency at least until the date shown on the bottle label when stored at 2 to 8°C. The reconstituted material may be stored at -20°C for six months or at 2 to 8°C for two weeks without loss of activity (in the absence of bacterial contamination).

PRESENTATION

Bottles of 2 mg (dried) HA 16

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C03HA16GB

April 2001