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RHMS/CK

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For the attention of The Research Director

Dear Sir,

L-Asparaginase

The Microbiological Research Establishment of the U.K. Government has prepared a unique L-asparaginase from Erwinia carotovora.

In view of the interest in this material for experimental work in cancer research many research institutions will wish to obtain supplies for laboratory purposes.

We are proud to announce that the Microbiological Research Establishment has entrusted the distribution of L-asparaginase for laboratory use only, to BDH Chemicals Ltd on an exclusive basis throughout the world with the exception of Europe, Greece and Turkey, where other arrangements apply.

We would ask you to note that this material should not be used for clinical trial purposes.

Technical data is given on the attached Product Information Sheet. L-asparaginase is supplied in ampoules containing a minimum of 1,000 I.U. at a price of 600/- per ampoule. If you require further advice on availability within your own country please write to us directly.

Yours faithfully,
BDH CHEMICALS LTD

R. H. M. Symons
Director

BDH Chemicals Ltd Poole England

PRODUCT INFORMATION

Title

Sheet No 174H/2·0

L-Asparaginase from *Erwinia carotovora*

While L-asparaginase is widely distributed in bacteria, only the enzymes from two species, *viz. Escherichia coli*[1] and *Serratia marcescens*[2], initially appeared to show useful levels of anti-tumour activity. More recently, however, L-asparaginase from strains of *Erwinia,* and especially from *Erwinia carotovora,* has been found to have higher specific activity than that from *E.coli,* and to be serologically distinct[3].

In C3H mice tumour formation was inhibited by intraperitoneal treatment with 500—600 I.U. per kg of L-asparaginase from *Erwinia carotovora,* four days after an injection of 3×10^5 cells of the lymphoma 6C3HED.

Safety tests in mice showed that doses of 200,000 I.U. L-asparaginase per kg could be given without any adverse clinical signs[3]. This compares very favourably with commercial preparations of the *E.coli* enzyme.

L-Asparaginase from *Erwinia carotovora,* prepared by the Microbiological Research Establishment, Porton, Salisbury, England, under contract to the Ministry of Health, is now commercially available through BDH Chemicals Ltd, for investigational use with laboratory research animals or for tests *in vitro.* The enzyme, in the form of a lyophilised powder, conforms to the following specification:

Specific activity:
The specific activity varies from 500—700 I.U. per mg protein, the precise figure being given on each ampoule. (1 International Unit ≡ 1μmol ammonia liberated per minute at 37°C in 0·05M borate buffer pH 8·5. Protein determined by the Lowry method.)

Homogeneity:
>90% by the following criteria:

Analytical ultracentrifugation combined with Refractometry using 7—9 mg protein per ml in 0·1 ionic strength phosphate buffer with 0·1M NaCl (pH 7·2—7·4) and examined after 34 min. at 59,780 rev./min. (phase plate 60°) on Spinco Model E. The preparation gives a symmetrical boundary at S_{20w} 7·3—7·5.

Electrophoresis on Cellogel in 0·05M glycine buffer pH 10·5 with a 12 cm bridge. Samples of 20—40 mg protein per cm are applied and 1mA per cm passed for 2 hours in a Shandon Electrophoresis tank. The strips are stained with naphthalene black 12B (as recommended in Cellogel instructions), then cleared and scanned with a Joyce Loebl Chromoscan.

Acrylamide Gel Electrophoresis using 5% gels at pH 3·8 with a reservoir buffer of pH 4·3. Samples of 0·1—0·2 mg protein in 10—50 μl of 5% sucrose are applied to the surface of each gel and the gels subjected to a current of 10 mA for 2—3 hours in a Shandon Disc Electrophoresis Apparatus. After staining with 1% naphthalene black 12B in 7% acetic acid the gels are de-stained and scanned for the presence of enzyme aggregates in a Joyce Loebl Chromoscan.

Stability:
The preparation is stable indefinitely at −20°C and for at least six months at 4°C.
BDH L-asparaginase is supplied in sealed ampoules containing a minimum of 1000 I.U.
(Each ampoule contains 0·29 mg NaCl to prevent the formation of aggregates).

References:
1. Marshburn, L.T. and Wriston, J.C., *Arch.Biochem.Biophys.,* 1964, **105,** 450.
2. Rowley, B. and Wriston, J.C., *Biochem.Biophys,Res.Commun.,* 1967, **28,** 160.
3. Wade, H.E. *et al, Lancet,* 1968, **ii,** 776.