

## **Purified Tissue Dissociation Enzymes: Past, Present, Future Challenges**

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The incorporation of purified neutral protease and class I & class II collagenase isolated from *C. histolyticum* culture supernatants into defined tissue dissociation enzyme (TDE) mixtures was in part responsible for the success of the Edmonton protocol. The enzyme composition of Liberase™ HI Purified Enzyme Blend (HI) was determined by using design of experiment methodology to optimize the concentrations of neutral protease and collagenase required to maximize recovery of islets from human pancreata. Additional benefits from this approach were reproducible preparation of TDE products, increased shelf life, and dramatic reduction in endotoxin contamination. In early reports, purified TDEs gave significantly higher islet yields than those obtained after tissue digestion with crude collagenase products.

A recent report by Lakey's group showed that the biochemical characteristics of the HI product has changed with new lots giving significantly lower human islet yields than earlier lots of product. The primary assays to assess collagenase activity in purified enzyme blend products use peptide substrates. These substrates preferentially detect intact or degraded class II collagenase. There is a strong need to improve enzymatic assays for collagenase activity since it plays a pivotal role for initiating the tissue dissociation process.

VitaCyte is developing purified TDE mixtures that contain collagenase isolated from *C. histolyticum* collagenase culture supernatants. A critical focus is to develop improved collagenase activity assays whose results correlate with those obtained from islet isolation from human pancreata. Two collagen degrading assays were developed using FITC-collagen fibrils as substrate. In the spectrophotometric assay, enzyme activity is detected by the release of A<sub>494</sub> protein into the supernatant after the intact collagen is removed by using a precipitating agent. In the fluorescence microplate assay, activity is detected by the increase of FITC fluorescence with time. The fluorescence microplate assay was shown to be superior to the spectrophotometric assay in detecting degraded forms of collagenase.

Further studies showed VitaCyte's purified collagenase is comparable to the purified collagenase used in Liberase HI after evaluation of these materials by physicochemical and enzymatic analysis. VitaCyte's preparation was also shown to be effective in tissue dissociation, with pre-ficoll porcine islet yields of > 5175 EIN/g of pancreas when market weight pigs (200-250 pounds) were used as the source of the organ.

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