Cytochrome b\textsubscript{5} (b\textsubscript{5}) was purified from liver microsomes from a single human subject using conventional techniques, including hydrophobic, anion-exchange, and hydroxylapatite adsorption chromatographies. Human b\textsubscript{5} is provided in 100 mM potassium phosphate buffer (pH 7.4), 0.1 mM EDTA, 0.1 mM DTT, and 20% glycerol.

♦ **Purity**

Purity has been determined by electrophoresis on 10% acrylamide gels run with the discontinuous buffer system. Human b\textsubscript{5} migrates as a single band with a molecular weight of 17.5 kDa (see Fig. 1, lane A). B\textsubscript{5} content is measured from the absolute oxidized spectrum using an extinction coefficient (E) of 117 mM\textsuperscript{-1} cm\textsuperscript{-1} at 413 nm.

**SDS-PAGE analysis of purified human liver cytochrome b\textsubscript{5}**

Lane A, cytochrome b\textsubscript{5} (0.5 µg);
Lane B, liver microsomes (10 µg);
Lane C, P450 Reductase (0.5 µg);

♦ **Reconstitution**

Addition of b\textsubscript{5} to a P450 reconstituted system (containing P450 enzyme, P450 reductase, and phospholipid) often results in metabolic properties (e.g., K\textsubscript{M}) more closely resembling those of intact liver microsomes. This is especially true with CYP2E1, which seems to require b\textsubscript{5} for efficient catalytic function. B\textsubscript{5} should be added to the P450 reconstituted system at a molar ratio of at least 4:1 (200 pmol b\textsubscript{5} : 50 pmol P450).

♦ **Storage**

Cytochrome b\textsubscript{5} should be stored @ -80°C. Avoid repeated freeze-thawing cycles.