Short Communication

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Microbial production of ketoreductases: Development of a novel high-throughput screening method

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Abstract

An assay method for detection of enantiospecific chiral alcohol was developed based on ketoreductase, enantio-selective alcohol oxidase and 2, 4-dinitrophenyl hydrazine (DNPH) reagent. The assay method was developed to check the conversion of 1-acetonapthone to either (S) or (R) specific 1-(1-napthyl) ethanol or its racemic mixture using ketoreductases. Further, estimation was done with the help of 2, 4- DNPH method. The resulting orange coloured chromogen showed a maximum absorbance at 560 nm. The assay was performed in 96 well microtiter plates and had a linear detection range from 0.05mM to 4mM. The method is found to be suitable for the detection of large numbers of crude samples and screening of ketoreductase producing strains in high-throughput manner.

Keywords: *High-throughput assay, Ketoreductase, Enantiospecific, Enantioselectivity, Alcohol oxidase.*

Introduction

Among various classes of enzymes, oxidoreductase represents a highly versatile class of biocatalyst currently used for the production of a wide variety of chemical and pharmaceutical products. Oxidoreductases are generally employed in whole-cell biotransformations or fermentation-based processes because they require expensive cofactors and coenzymes (e.g. NAD(H) or NADP(H)) to donate or accept the chemical equivalents for