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Superoxide Generating System

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Introduction

There are several chemical ways of generating superoxide anion radical ($\cdot O_2^-$); in the biochemical and medical research fields an enzymatic method using xanthine oxidase is widely used (Figure 1).

Protocol

Reagents used are Hypoxanthine solution (1 mM) and xanthine oxidase, Fenton reaction.

Procedure

1. Take hypoxanthine solution (1 mM). For cell culture, use phosphate-buffered saline or foetal calf serum-free medium instead of usual culture medium.
2. Add xanthine oxidase (0.01-100 munits/mL).
3. Incubate for an appropriate time determined experimentally.
4. For cell culture, remove the hypoxanthine solution by aspiration and add the culture medium. The cells are harvested when ready.

Comments

If xanthine oxidase is used H_2O_2 and $\cdot O_2^-$ will be produced. If transition metal ions or their redox active complexes are also present hydroxyl radical will be produced (Fenton reaction). To remove H_2O_2 add catalase to the reaction.

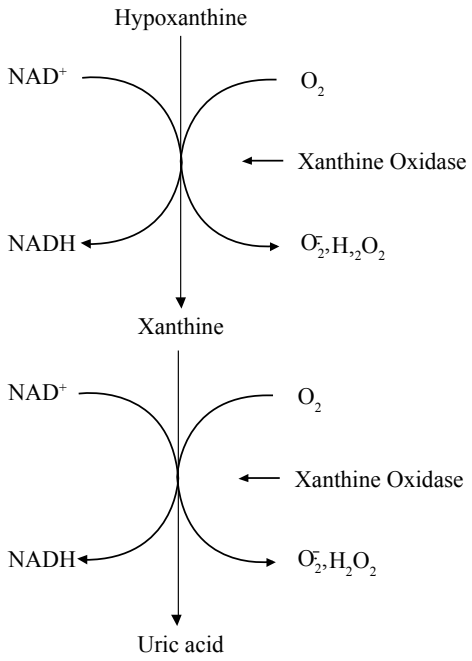

Figure 1: Generation of superoxide. ($\cdot O_2^-$) and hydrogen peroxide (H_2O_2) from hypoxanthine and xanthine oxidase.

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