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Fixation of microorganisms with paraformaldehyde for TLR stimulation.

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Method Article

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Abstract

Toll-like receptors (TLRs) are part of the family of pattern recognition receptors (PRRs) that evolved to recognize pathogen-associated molecular patterns (PAMPs). A variety of PAMPs have been identified, from various microbial components to viral molecules. *E.coli* (*Escherichia coli*) has been widely used as a prototype Gram-negative bacteria with the capacity to stimulate TLRs, in particular to elicit TLR4-dependent immune responses. This receptor mainly recognizes an essential component of the outer membrane of Gram-negative bacteria: lipopolysaccharide (LPS). In vitro stimulation of immune cells for cytokine production can be achieved with the use of commercially-available purified TLR agonists, including LPS. Here we show how to prepare an alternative stimulator of TLR4 in a very fast an inexpensive way, paraformaldehyde-fixed preparations of *E. coli.* This reagent is a powerful tool for the *in vitro* activation of TLR4 that can be quantified and therefore used in defined amounts. Moreover, it is a safe reagent (the *E.coli* cells are fixed) and it is easy to prepare in large quantities and to store. Other microorganisms such as yeasts can also be fixed in this way.

Introduction

This protocol can be used to fix microorganisms such as bacteria (*E. coli* for example) and yeasts (such as *C. albicans*).

Reagents

Microbial culture in liquid medium;

4% w/v solution of paraformaldehyde (PFA) in PBS;

PBS medium 1x.

Equipment

Shaker at the optimal temperature for the growth of the particular microorganism.

Centrifuge;

Nanodrop;

Procedure

1. Grow an O/N culture (approximately 200mL) in liquid medium (bacteria or yeast) in a shaker at the optimal temperature for the particular microorganism.

2. Split the culture in 10 tubes, (each with 20mL) and centrifuge to pellet the cells (1minute at maximum speed).

3. Ressuspend each pellet in 20mL of PBS 1x.

4. Repeat the centrifugation and wash steps twice.

5. Centrifuge again and ressuspend the cells in 1mL of 4% PFA solution.

NOTE! PFA is toxic, perform this step in a fume hood.

6. Incubate 5 minutes at RT.

7. Centrifuge again and ressuspend the cells in 1mL of PBS.

8. Combine all the tubes into one cell suspension.

9. Read optical density at 600 nm (OD600) in a Nanodrop spectrophotometer.

NOTE! Mix the cell suspension well before measuring.

10. Calculate the concentration of the bacterial or yeast suspension.

(This can be done using a standard curve previously obtained by measuring the OD600 of a series of dilutions of the microbial culture and by plating those cultures in agar plates and counting the cell numbers).