

**EXPERIMENTAL METHODS
IN MODERN BIOTECHNOLOGY**

Editors

Ibrahim Ali Noorbacha

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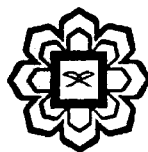


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Mohd Rushdi Abu Bakar, Zoltan Karman Nagy, Ali Nauman Saleemi and Christopher David Rielly

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Methods for Screening the Potential Natural Compound for Treating Metabolic Disorder Diseases: Anti-Inflammatory and Griess Assay [Nitric Oxide (NO) Measurement]

Azura Amid, Sulawatie Semail, Wan Dalila Wan Chik and Hammed Ademola Monsur

1. Introduction

Nitric oxide (NO) is an important messenger in many biological systems (Bredt and Snyder, 1995) that initiates host defense. Thus, activation of the immune system is believed to be associated with an increase in macrophage NO production (Stuehr and Marletta, 1985). Most of the time NO is oxidized to nitrite (NO_2^-) and nitrate (NO_3^-), make it suitable to be measure quantitatively by spectrophotometric measurement. Griess Reaction is responsible during the NO measurement, which is based on the enzymatic conversion of nitrate to nitrite by nitrate reductase. There are two steps diazotization reactions. First, acidified NO_2^- produces a nitrosalting agent that reacts with sulfanilic acid to produce diazonium ion. Then, this ion coupled to *N*-(1-naphthyl) ethylenediamine to form chromophoric azo-derivative that absorbs light at 540 nm (Miles *et al*, 1996).

RAW 264.7 cell line is a macrophage cell. According to Jung and co-workers (2009) macrophages are major immune cells in the innate immune system. The activation of macrophages plays a key role in inflammatory responses to infection with pathogen and able to kill pathogens directly by phagocytosis and indirectly by various pro-inflammatory mediators such as nitrogen species and pro-inflammatory cytokines (TNF- α and IL-1 β) and prostaglandin E_2 (PGE_2). In-vitro inflammatory induction will be achieved by treating these macrophages with lipopolysaccharide (LPS).

2. Objective

This chapter covers the method that was modified from Promega Technical Buletin (www.promega.com) and R & D System Technical Bulletin (www.rndsystems.com) but Griess in 1879 is actually the one who described the Griess assay in one of his publication (Griess, 1879).