EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

Ibrahim Ali Noorbatcha

Mohamed Ismail Abdul Karim

Hamzah Mohd Salleh

IIUM Press

EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Editors

Ibrahim Ali Noorbatcha
Mohamed Ismail Abdul Karim
Hamzah Mohd Salleh

IIUM Press

Published by:
IIUM Press
International Islamic University Malaysia

First Edition, 2011

Perpustakaan Negara Malay Cataloguing-in-Publication Data

Ibrahim Ali Noorbatcha, Mohamed Ismail Abdul Karim and Hamzah Mohd Salleh
Experimental Methods in Modern Biotechnology

ISBN: 978-967-0225-86-9

Member of Majlis Penerbitan Ilmiah Malaysia – MAPIM
(Malaysian Scholarly Publishing Council)

Printed by:
IIUM PRINTING SDN. BHD.
No. 1, Jalan Industri Batu Caves 1/3
Taman Perindustrian Batu Caves
Batu Caves Centre Point
68100 Batu Caves
Selangor Darul Ehsan

CONTENTS

Chapter 3. 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

Chapter 4. Factors Affecting Enzyme Assays

Hamzah Mohd Salleh

Chapter 5. Techniques of Extraction and Purification of Fucoxanthin from Brown Seaweeds

Irwandi Jaswir, Dedi Noviendri, Hamzah Mohd Salleh, Muhammad Taher and Kazuo Miyashita

Chapter 6. Fundamentals of Proximate Analysis in Food Products

Irwandi Jaswir and Asiyanbi-Hammed Tawakalit Tope

Chapter 7. Fish Gelatin Production: Extraction Method and Quality Analysis

Irwandi Jaswir, Hammed A. Monsur and Hamzah M. Salleh

Chapter 8. Application of Fourier Transform Infrared Spectroscopy Edible Fats and Oils Analysis

Mohamed Elwathig Saeed Mirghani

Chapter 12. Response Surface Methodology (RSM) Design for Bioreactor Operation

Maizirwan Mel and Najiah Nadir

Chapter 13. Screening Natural Compounds for Antibacterial Activity by Disc Diffusion Method

Raha Ahmad Raus

CHAPTER 3

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-1β) 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).

From: Experimental Methods In Modern Biotechnology
Edited by: *I. A. Noorbatch, H. M. Salleh and M. I. A. Karim. IIUM Press, KL, Malaysia*