Sep 04, 2018 Version 1

In vitro α-amylase inhibitory assay V.1

DOI
dx.doi.org/10.17504/protocols.io.s9meh46

Maira Rubi, Maira Rubi Segura Campos[1]

[1]Universidad Autónoma de Yucatán

Maira Rubi Segura Campos

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS

DOI: https://dx.doi.org/10.17504/protocols.io.s9meh46

Protocol Citation: Maira Rubi, Maira Rubi Segura Campos 2018. In vitro α-amylase inhibitory assay. **protocols.io https://dx.doi.org/10.17504/protocols.io.s9meh46**

Manuscript citation:
Dineshkumar B, Mitra A, Manjunatha M. Studies on the anti-diabetic and hypolipidemic potentials of mangiferin (xanthone glucoside) in streptozotocin-induced type 1 and type 2 diabetic model rats. Int J Adv Pharm Sci. 2010; 1: 75-85.

License: This is an open access protocol distributed under the terms of the **Creative Commons Attribution License**, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working
We use this protocol and it's working

Created: September 04, 2018

Last Modified: September 04, 2018

Protocol Integer ID: 15373

Keywords: amylase inhibitory activity, pancreatic amylase in tri, pancreatic amylase, amylase, inhibitory assay, solvent with enzyme, enzyme, starch, solvent without enzyme, aqueous extract, inhibitory activity, acetic acid, ml of aqueous extract, ml of acetic acid, test sample without enzyme, substrate solution, assay

Abstract

The assay was carried out following the protocol reported by Dineshkumar et al. [8]. Starch (2 mg) was suspended in a tube containing 0.2 mL of 0.5 M Tris-HCl (Sigma-Aldrich, USA) buffer (pH 6.9) with 0.01 M calcium chloride as substrate. The tube was boiled for 5 min and then preincubated at 37 °C for 5 min. Plant aqueous extract (1 mg) was dissolved with 1 mL of 0.1% of dimethyl sulfoxide in order to obtain a concentration of 1,000 µg/mL; then 0.2 mL of aqueous extract was added to the tube containing the substrate solution, 0.1 mL of porcine pancreatic amylase in Tris-HCl buffer (2 U/mL) was also added, and incubated for 10 min at 37 °C. Finally, the reaction was stopped with 0.5 mL of acetic acid (50% v/v) and centrifuged 5 min at 1,811 × g and 4 °C. The assay was performed in triplicate. The a-amylase inhibitory activity was calculated using the formula $(Ac^{+}) – (Ac^{-}) – (As - Ab)/(Ac^{+}) – (Ac^{-}) \times 100$, where Ac^{+}, Ac^{-}, As, Ab are defined as the absorbance (595 nm) of 100% enzyme activity (only solvent with enzyme), 0% enzyme activity (only solvent without enzyme), test sample (with enzyme), and a blank (a test sample without enzyme), respectively.

Troubleshooting