



# Supplementary Materials of Nano-Encapsulated Melatonin: A Promising Mucosal Adjuvant in Intranasal Immunization against Chronic Experimental *T. gondii* Infection

## Melatonin Spectrophotometric Assay

Stock solution of melatonin was prepared in ethanol (1 mg/mL). Selected volumes of stock solution were diluted in water (0.005–0.025 mg/mL). UV scanning was done using UV Spectrophotometer (Cary 60-UV-VIS, Agilent technologies, (Koyoto, Japan) from 200–400 nm. The wavelength maxima ( $\lambda$  max) was detected from the spectrum (Figure S1). Absorbance of each concentration was measured at  $\lambda$  max detected (278 nm). Measurements were done in triplicate; calibration curve was constructed with  $R^2$  value of 0.9998 (Figure S2).

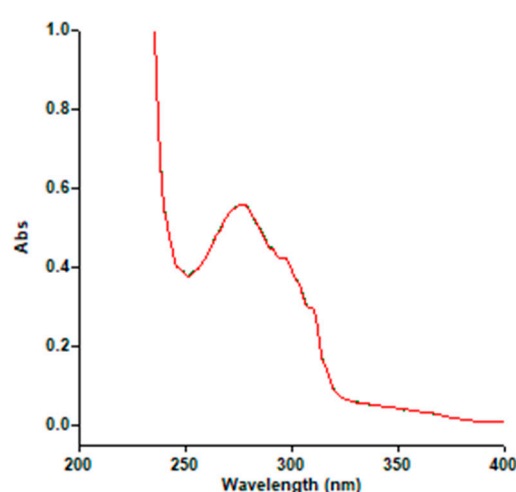


Figure S1. Melatonin UV spectrum.

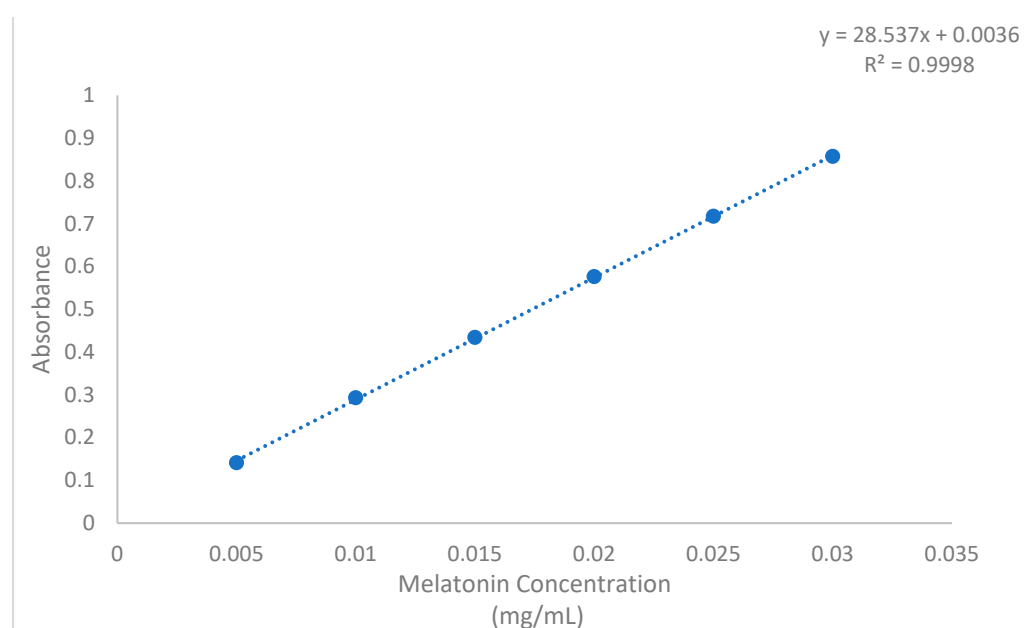


Figure S2. Calibration curve of Melatonin.