ABSTRACT
Cholesterol imbalance is implicated in a variety of neurodegenerative diseases including Alzheimer's Disease, Huntington's Disease, and Multiple Sclerosis. Excess cholesterol is removed from the brain via the hydroxylation of C24 on cholesterol by CYP46 enzyme, a highly conserved member of the cytochrome P450 family, to the more soluble 24(S)-Hydroxycholesterol (24-OHC) which is able to cross the blood brain barrier. Levels of 24-OHC in the cerebral spinal fluid (CSF) may give insight into the homeostasis of cholesterol within the central nervous system, and may serve as a valuable biomarker for neurodegenerative disease. We have developed a competitive colorimetric ELISA for the measurement of 24-OHC that can be used in both in vivo and in vitro studies. The assay is highly specific to 24-OHC, exhibiting less than 0.02% cross-reactivity to cholesterol, 22-hydroxycholesterol, 25-hydroxycholesterol, 27-hydroxycholesterol and dehydroepiandrosterone (DHEA). The assay returns 24-OHC levels in CSF consistent with levels reported in the literature, and is also suitable for tissue homogenates and tissue culture supernatants, requiring 100 µL or less of sample volume. The assay has a dynamic range from 0.78 to 100 ng/mL and a time to answer of 2 hours, making it a convenient alternative to more labor- and instrument-intensive methods such as GC-MS, LC-MS and HPLC-MS.

BACKGROUND
The homeostasis and trafficking of cholesterol is an essential component of both the central and peripheral nervous system in the maintenance of neuronal tissues,1,2 and disturbances in this homeostasis may be due to the onset of various neurological diseases such as Alzheimer’s Disease (AD), Huntington’s Disease (HD) and multiple sclerosis (MS).3,4,5 Apolipoprotein E and Cyp46 (also known as 24S-Cholesterol Hydroxylase) are both important in the homeostasis of cerebral cholesterol6 and thus are of clinical interest in understanding the relation of these molecules to the pathogenesis of these, and potentially other, neurodegenerative diseases.

24-OHC, an enzymatically-generated side chain-hydroxylated derivative of cholesterol, is a pivotal marker in the study of cerebral cholesterol homeostasis. Cholesterol is unable to cross the blood-brain barrier (BBB) however, Cyp46 enzyme converts cholesterol to the more soluble 24-OHC, and this hydroxylated form of cholesterol is able to cross the BBB.2,4 This conversion allows for the reduction of cholesterol in the brain and the efflux of 24-OHC from the brain into cerebral spinal fluid and blood. The flux of 24-OHC has been observed in patients with a variety of neurodegenerative diseases.3,4,5 In the instance of Alzheimer’s disease, the change in 24S-hydroxycholesterol concentrations may be indicative of different pathogenetic mechanisms and/or the progression of the disease.3 As in the case of multiple sclerosis, concentrations of 24-OHC have been shown to decrease, likely due to the loss of neuronal cells responsible for the synthesis.3

Current 24-OHC measurements are performed using GC-MS, LC-MS and HPLC-MS methodologies, all of which carry high initial instrumentation costs and routing maintenance. We have developed a competitive colorimetric ELISA for the measurement of 24-OHC for use in both in vivo and in vitro studies.

Figure 2: Typical standard curve produced using 24-OHC ELISA kit standards.