In contrast to extracellular plaque and intracellular tangle pathology, the presence and relevance of intraneuronal Aβ in Alzheimer's disease (AD) is still a matter of debate. Human brain tissue offers technical challenges such as post-mortem delay and uneven or prolonged tissue fixation that might affect immunohistochemical staining. In addition, previous studies on intracellular Aβ accumulation in human brain often used antibodies targeting the C-terminus of Aβ and differed strongly in the pretreatments used. To overcome these inconsistencies, we performed extensive parametrical testing using a highly specific N-terminal Aβ antibody detecting the aspartate at position 1, before developing an optimal staining protocol for intraneuronal Aβ detection in paraffin-embedded sections from AD patients. To rule out that this antibody also detects the β-cleaved APP C-terminal fragment (β-CTF, C99) bearing the same epitope, paraffin-sections of transgenic mice overexpressing the C99-fragment were stained without any evidence for cross-reactivity in our staining protocol. The staining intensity of intraneuronal Aβ in cortex and hippocampal tissue of 10 controls and 20 sporadic AD cases was then correlated to patient data including sex, Braak stage, plaque load, and Apolipoprotein E (ApoE) genotype. Interestingly, the presence of one or two ApoE4 alleles strongly correlated with an increased accumulation of intraneuronal Aβ peptides. Given that ApoE4 is a major genetic risk factor for AD and is involved in neuronal cholesterol transport, it is tempting to speculate that perturbed intracellular trafficking is involved in the increased intraneuronal Aβ aggregation in AD.