Graphene-based quantum dots (GQDs) are attractive fluorophores due to their excellent photoluminescence properties, water solubility, low cost, and low toxicity. However, the lack of simple, efficient, and environmental-friendly synthesis methods often limits their biological applications. Herein, we explore a novel, one-pot, green synthesis approach for the fabrication of fluorescent GQDs without involving any harsh reagents. Graphene oxide is used as a precursor, and a 2 h hydrothermal synthesis is carried out with assistance of hydrogen peroxide; no further post purification steps are required. The effects of reaction conditions on the characteristics of GQDs are comprehensively investigated. The as-synthesized GQDs show a high photostability and excellent biocompatibility as revealed by cell viability assays for three different cell lines, namely, macrophages, endothelial cells, and a model cancer cell line. The detailed studies of cellular uptake mechanisms suggest that for all of the three cell lines the major internalization route for GQDs is caveolae-mediated endocytosis followed by clathrin-mediated endocytosis at a less extent. Our results demonstrate the great potential of the as-synthesized GQDs as fluorescent nanoprobes. The study also provides unique insight into the cell–GQDs interactions, which is highly valuable for bioimaging and other related applications such as diagnostics and drug delivery.