Stabilized plasmid lipid nanoparticles (SPLPs) have been developed to overcome the low stability issue of cationic liposomes, however, SPLPs that are too stable result in unsatisfactory transfection efficiency. In this article, we prepared enzyme-responsive SPLPs (eSPLPs) composed of 1,2-dioleoyl-3-trimethylammonium-propane (DOTAP), 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine (DOPE), and mPEG-GLFG-K-(C16)2, a PEG lipid with an enzymatically-cleavable linker (glycine-phenylalanine-leucine-glycine, GFLG). eSPLPs were successfully prepared with a pDNA encapsulation efficiency of over 80%, using the detergent dialysis method. The PEG shell stabilized the eSPLPs and maintained a hydrodynamic diameter of 200 nm, however, in endosomal conditions, cathepsin B degraded the GFLG linker, decoating the PEG shell from the eSPLPs. Decoated eSPLPs were rapidly destabilized, and induced endosomal disruption in order to the encapsulated pDNA to escape into the cytosol. Owing to the cathepsin B-responsive destabilization, the eSPLPs showed a 10 to 100-fold higher transfection efficiency than normal SPLPs, which was confirmed using luciferase assay. These results suggest that eSPLPs are a promising candidate for use as a practical gene delivery carrier, with both stability and high transfection efficiency for future in vivo applications.