Protein tyrosine nitration (PTN) is a post-translational modification that is related to several acute or chronic diseases. Although nitrated proteins as markers of oxidative stress are confirmed by immunological assays in various affected cells or tissues, it is not known how many different types of proteins in living cells are nitrated. Since PTN is a low-abundance post-translational modification, development of an effective enrichment method for nitrated proteins is needed to detect nitrated peptides or proteins from the limited amount of pathophysiological samples. Recently, we developed an enrichment method using specific chemical tagging. Nitroproteome profiling using a novel chemical tagging and mass spectrometry was validated by model proteins. Furthermore, we successfully identified numerous nitrated proteins from the various biological systems including embryonic stem cell, Huh7 human hepatoma cell line and yeast.