Glucosinolates (GSLs) are sulfur-containing secondary metabolites that are abundantly present in Brassicaceae plants family and in the whole order of Capparales. Glucosinolate components of radish, such as glucoraphenin, a glucosinolate that can be hydrogenated to form sulforaphene, has been identified as being particularly potent in its anticancer related biological activities. The main objective of this study was to identify and quantitatively profile the GSLs content in roots of various germplasm collections along with their morphological characters in National Agrobiodiversity Center (NAC), Jeonju, Korea. We have also examined the spatial distribution of GSLs in the leaves and roots using three samples. We analyzed the morphological characters and GSLs contents of 532 accessions and seven commercial cultivars of Raphanus sativus var. sativus root obtained from 26 different countries and cultivated at the experimental field of NAC. Six GSLs (Glucoraphasatin, Glucobrassicin, Glucoraphenin, Glucoberteroin, Glucoraphanin, Glucobrassicin) were identified and glucoraphasatin was the most abundant, representing over 90%, of the GSLs studied. Among the germplasm collections, three accessions exhibited over 1,000 mg·kg\(^{-1}\) dry weight (DW) total GSLs and the average was 518 mg·kg\(^{-1}\) DW. The total GSLs contents in commercial cultivars ranged from 177 to 565 mg·kg\(^{-1}\) DW. A study of the spatial distribution of the GSLs showed that the GSLs content increase from top to bottom in both leaves and roots of radish. Accessions “IT102431” (1,106 mg·kg\(^{-1}\) DW), “IT102468” (1,022 mg·kg\(^{-1}\) DW) and “IT102384” (1,011 mg·kg\(^{-1}\) DW), the first two originated from Turkey and the later a seashore wild radish (gaet-mu) from Jeju, South Korea, represented the highest total GSLs. These accessions could be potential candidates to develop new varieties of radish with high content of GSLs.