Gold nanoparticles (AuNPs) are increasingly studied for cancer treatment purposes, as they can potentially improve both control and efficiency of the treatment. Intensive research is conducted in vitro on rodent and human cell lines to objectify the gain of combining AuNPs with cancer treatment and to understand their mechanisms of action. However, using nanoparticles in such studies requires thorough knowledge of their cellular uptake. In this study, we optimized single particle ICPMS (sp-ICPMS) analysis to qualify and quantify intracellular AuNP content after exposure of in vitro human breast cancer cell lines. To this aim, cells were treated with an alkaline digestion method with 5% TMAH, allowing the detection of gold with a yield of 97% on average. Results showed that under our experimental conditions, the AuNP size distribution appeared to be unchanged after internalization and that the uptake of particles depended on the cell line and on the exposure duration. Finally, the comparison of the particle numbers per cell with the estimates based on the gold masses showed excellent agreement, confirming the validity of the sp-ICPMS particle measurements in such complex samples.