1. Selection of target proteins to be quantified

2. Selection of target peptides to be quantified

3. Synthesis of unlabeled and labeled peptides

4. AAA and LC-MS/MS optimization

5. Preparation of calibration curve

6. Preparation of protein sample

7. Preparation of peptide sample:
   1) Denaturation
   2) Reduction/alkylation
   3) Enzymatic digestion
   4) Mix with stable isotope labeled peptide mixture
   5) Acidification
   6) Desalting

8. Analysis by LC-MS/MS

9. Calculation of protein expression level