The following procedure describes the analysis of samples obtained from in-gel digestion by a quadrupole time-of-flight tandem mass spectrometer equipped with an orthogonal matrix-assisted laser desorption/ionization ion source (oMALDI-QqTOF). The mass spectrometric data are used for the identification of proteins.

**Procedure**
1. Redissolve the dried protein digests in 5 to 10 µl trifluoroacetic acid, 0.1% (0.1% TFA).
2. Apply samples onto ZipTip pipette tips. Wash ZipTips three times with 20 µl 0.1% TFA each time.
3. Elute peptides with 1 µl 2, 5-dihydroxybenzoic acid (DHB) matrix solution directly onto a clean matrix-associated laser desorption ionization (MALDI) sample plate. Air-dry the plate or dry plate in a vacuum for use in mass spectrometric analysis.
4. Analyze samples on a QSTAR Pulsar-i quadrupole time-of-flight tandem mass spectrometer equipped with an oMALDI ion source and a 30 Hz nitrogen laser.
5. Acquire mass spectometry (MS) and tandem mass spectometry (MS/MS) data. Set appropriate laser repetition rate (from 5 to 20 Hz, depending on the amount of sample and peak intensity). Adjust acquisition time to obtain spectra with a sufficient number of peaks of adequate intensity.
6. In the MS/MS scan mode, precursor ions are selected in quadrupole Q1 and fragmented in the collision cell (q2), using argon as the collision gas. For each precursor ion, set the collision energy initially using a rule of 0.05 V/ Da, and then adjust it to obtain the desired degree of fragmentation.
7. Identify proteins by using MS and MS/MS data to search NCBI nonredundant mouse or mammalian protein databases using various software packages.

**Reagents and Materials**
- Trifluoroacetic acid, 0.1% (0.1% TFA): AfCS Solution Protocol PS00000503
- ZipTip pipette tips: Millipore; catalog no. ZTC18S960
- 2, 5-Dihydroxybenzoic acid (DHB) matrix solution: Agilent; catalog no. G2039A
- Argon: Matheson Tri-Gas, Inc.; catalog no. 1901112-1
- QSTAR Pulsar-i quadrupole time-of-flight tandem mass spectrometer: Applied Biosystems/MDS Sciex