

## ESI-MS Analysis of Proteins (Code A):

### 1. General Procedure

The mass spectra are acquired using the QTrap linear ion trap mass spectrometer (AB/MDS Sciex, Toronto, Canada). The protein sample is injected into the sample loop and delivered to the mass spectrometer using 65% acetonitrile/0.1% formic acid in water at 20  $\mu\text{l}/\text{min}$ . The curtain gas is set at 25 (arbitrary units) while ion source gas 1 is set at 20. The ion spray voltage is set at 5500 V and declustering potential is set at 20 V. The scan rate is 4000 amu/s with step size of 0.12 amu.

### 2. Mass Accuracy and Resolution

The mass accuracy is typically 0.5 Da for m/z value. The resolution is around 2000.  $\text{Resolution} = M/\Delta M$

### 3. How to Read the Spectrum?

The ESI mass spectrum normally consists of consecutive peaks of multiply charged molecular ions obtained through protonation  $(M+zH)^{z+}$ . The average molecular weight of the protein can be calculated based on the measured mass-to-charge ratio (m/z) and the number of charges (z).

For example:

$$\text{MW}_1 = (m/z) \times 10 - 10 \text{ (10 positive charges)}$$

$$\text{MW}_2 = (m/z) \times 11 - 11 \text{ (11 positive charges)}$$

$$\text{MW}_3 = (m/z) \times 12 - 12 \text{ (12 positive charges)}$$

The average MW =  $(\text{MW}_1 + \text{MW}_2 + \text{MW}_3 + \dots + \text{MW}_n) / n$

[See a representative ESI spectrum of protein .](#)