HER2-SRM analysis was performed on a liquid chromatograph (LC) (Waters ACQUITY UPLC M-Class System) connected to a triple quadrupole mass spectrometer (Thermo TSQ-Altis).

An LC gradient was use used to elute peptides. The flow phase A was water with 0.1% formic acid (Thermo Scientific, LS118), and the flow phase B was acetonitrile with 0.1% formic acid (Thermo Scientific, LS120). The chromatographic column set included a trap column (nanoEase MZ Symmetry C18 Trap Column, 100A, 5 µm 180 µm x 20mm) and an analytical column (nanoEase MZ HSS T3 Column, 100Å, 1.8 µm, 100 µm x 100 mm).

Peptides were eluted into the mass spectrometer using the following gradient: loaded onto trap column for 5 min with buffer A at a flow rate of 5 µl/min and eluted with buffer B using a step gradient at 800 nl/min. Buffer B was increased from 1–25% (8 min), 25–50% (7 min), and 50–95% (3 min). Finally, the column was cleaned with buffer B for 6 min and equilibrated with buffer A for 4 min.

Mass spectrometry method: Thermo TSQ-Altis mass spectrometer was operated in positive NSI mode and were used for the SRM assays: Q1(FWHM):0.7, Q3(FWHM): 0.7, dwell time: 100 ms, electrospray voltage: 2.3 kV, collision gas: 2 mTorr. The precursor ions for the light and heavy peptides are m/z 483.748 and 488.752. The fragment ions for the light and heavy peptides and their corresponding optimized collision energy are m/z 409.218 (17V)/538.261 (17V)/625.294 (17V) and 419.227 (17V)/548.270 (17V)/635.302 (17V), respectively.