

Claims

1. A method for quantification of at least one MHC peptide ligand on a cell, comprising
 - a) preparing a cell lysate from cells presenting said at least one MHC peptide ligand from a biological sample comprising cells to obtain a preparation A,
 - b) determining a cell count of said preparation A,
 - c) adding a pre-determined amount of said at least one MHC peptide ligand labelled with a first isotope in an MHC complex to be quantified to said preparation A ("spiking I") to obtain a preparation B,
 - d) isolating the at least one MHC peptide ligand from the preparation A and the at least one MHC peptide ligand labelled with a first isotope from said preparation B to obtain a peptide eluate,
 - e) adding a pre-determined amount of the at least one MHC peptide ligand labelled with a second isotope to be quantified to said peptide eluate ("spiking II") to obtain a preparation C, wherein the first isotope is different from the second isotope,
 - f) performing a mass spectrometry analysis on said at least one MHC peptide ligand in the preparation C to generate at least one
 - fa) signal for said at least one MHC peptide ligand labelled with the first isotope isolated from the preparation B to calculate efficiency of said isolating,
 - fb) signal for the pre-determined amount of said at least one MHC peptide ligand labelled with the second isotope as added to the preparation C, and
 - fc) signal for said at least one MHC peptide ligand from said preparation A,
 - f1) performing a mass spectrometry analysis on serially titrated amounts of said at least one MHC peptide ligand labelled with a third isotope and the pre-determined amount of said at least one MHC peptide ligand labelled with a fourth isotope as an internal standard to generate
 - f1a) signal for the serially titrated amounts of said at least one MHC peptide ligand labelled with a third isotope, and
 - f1b) signal for the pre-determined amount of said at least one MHC peptide ligand labelled with a fourth isotope, wherein the third isotope is different from the fourth isotope,
 - f2) plotting a ratio of the signal of f1a)/the signal of f1b) to generate a calibration curve, wherein a lower limit of quantitation (LLOQ) is determined when the ratio of the signal of f1a)/the signal of f1b) deviates from linearity in the calibration curve, and;
 - g) quantifying said at least one MHC peptide ligand in the preparation A based on a comparison of the signals as obtained in f) with
 - ga) the cell count as obtained,
 - gb) the pre-determined amount of said at least one peptide-MHC ligand labelled with the first isotope and/or peptide-MHC ligand complex labelled with the first isotope to be quantified as added to the preparation B, and
 - gc) the pre-determined amount of at least one MHC peptide ligand labelled with a second isotope to be quantified as added to the preparation C, wherein the quantifying in g) comprises selecting a ratio of the signal of fc)/the signal of fb) at or above the LLOQ and calculating the copy number of the at least one MHC peptide ligand per cell based on the selected ratio in the calibration curve.
2. The method according to claim 1, wherein said at least one MHC peptide ligand is selected from a tumor associated peptide (TAA) or disease associated peptide (DAA).

3. The method according to claim 1, wherein said biological sample comprising cells is selected from a tissue sample, a blood sample, a tumor sample, or a sample of an infected tissue.
4. The method according to claim 1, wherein the preparing cells comprises enzymatic digestion of tissues, and/or cellular lysis.
5. The method according to claim 1, wherein said cell count is determined using a method selected from counting cell nuclei, photometric DNA-determination, fluorimetric DNA-determination, or quantitative PCR.
6. The method according to claim 1, further comprising determining the amount of at least one type of HLA-molecule in said preparation A.
7. The method according to claim 1, wherein the isolating comprises using chromatography.
8. The method according to claim 1, further comprising selecting overpresented, overexpressed and/or tumor-specific MHC peptide ligands for analysis.
9. The method according to claim 1, wherein said method is capable of being performed and/or is performed on a high-throughput basis.
10. The method according to claim 1, wherein said method consists of said a) to g).
11. The method according to claim 1, wherein said biological sample is derived from one individual, or from a group of individuals suffering from the same medical condition.
12. The method according to claim 11, further comprising generating a personalized MHC ligand profile or a personalized disease-specific MHC ligand profile, based on said MHC peptide ligands as quantified.
13. The method according to claim 1, wherein the isolating comprises using affinity chromatography.
14. The method according to claim 1, wherein said biological sample is derived from one individual.
15. The method according to claim 11, further comprising generating a personalized MHC ligand profile based on said MHC peptide ligands as quantified.
16. The method according to claim 11, further comprising generating a personalized disease-specific MHC ligand profile based on said MHC peptide ligands as quantified.