Preparation of MS/MS samples after in-gel digestion

The sample will be dissolved in water solution (because acetonitrile should be dried), 40 ul.

de-salting with Zip-Tips:

Prepare following reagents: each 1 ml is enough
wetting solution 100% acetonitrile(ACN)
sample (pH <4)
Equilibration, and wash solution 0.1% TFA in milli-Q
Elution solution, 0.1%TFA/50% ACN

Set zip-tip on pipetteman
Transfer 10 ul of each solution (wetting, equilibration, wash(20 ul), elution solution, and sample) into new tubes
pipette in and out wetting solution (10 ul) several times
pipette in and out equilibration solution (10 ul) several times
pipette in and out sample (10 ul) several times
pipette in wash solution and discard, repeat 2 times
pipette in and out 2 ul of elution solution several times

We have two types of target plates, one is normal, and the other is "anchorchip".
anchorchip is adopted to sensitive experiments (such as low concentration of sample)
When we use anchorchip, sample spotting should be done in different way than normal.

Sample spotting on anchorchip:
(p15 of anchorchip manual)

prepare following reagents.
matrix solution: HCCA(alpha-cyano-4-hydroxy cinnamic acid) 0.2 mg/ml in ACN 1 ml
  vortex, centrifuge, and use supernatant.
Analyte solution=ACN:TFA solution=1:2 1 ml
TFA solution: 0.1% TFA 1 ml

Mix matrix and analyte premix (5:1) and spot onto 400 um or 600 um anchor
leave it until the sample dry

A dense layer of tiny well-separated crystals covering the anchor should be visible under microscopic inspection. If a significant number of crystals are located outside the anchor area, decrease the water content in the premix or recrystallize the sample. If the density of crystals is too low, increase the HCCA concentration.