

**Manual work-up**

1. Take out the filter disc from the Capitainer device using the Capitainer puncher or do it manually by using a tweezer. Put in wells on a 96-well protein filtration plate.
2. Prepare blanks, QCs and calibrators in proper wells.
3. In plates with calibrators and QCs the calibrators are placed in wells A1-8 and, blank and QCs in wells B1-8.
4. Put the extraction plate on top of the collection plate.
5. The discs are wetted by adding 20  $\mu\text{L}$  of water, wait for 5 min.
6. Add 200  $\mu\text{L}$  of IPA extraction solvent (containing internal standard).
7. Add 10  $\mu\text{L}$  of proper calibrator solution to the calibrators and another 10  $\mu\text{L}$  of IPA to unknowns and controls.
8. Shake at 700 rpm speed for 30 min.
9. Centrifuge down the liquid phase to the 96-well collection plate positioned below.
10. Add 200  $\mu\text{L}$  of IPA solvent.
11. Shake for 15 min.
12. Centrifuge down the liquid phase to the same wells positioned below.
13. Cover plate with cover bed.
14. Shake gently for 1 min.
15. Extracts are ready for instrument autosampler