XBridge C18 high pH (10) fractionation protocol

- Column – XBridge C18, 250 x 4.6 mm, 5 µM with 4.6 x 20 mm guard column (Waters, Milford, MA).
- Mobile phases – solvent A: 10 mM ammonium formate, pH 10; and solvent B: 10 mM ammonium formate, pH 10, 90% acetonitrile.
- Sample separation was performed at a flow rate 0.5 mL/min using the following 5 step gradient: initial 10 min from 100 % solvent A to 5 % solvent B; next 60 min, step 2, from 95% solvent A to 35% solvent B; the following 15 min, step 3, increasing concentration of solvent B to 70%; then, step 4, 10 min at 70% solvent B; and step 5, 100% of solvent A for 15 min.
- The sample injection volume was 900.0 µL and the amount of injected peptides was 300.0 µg. The tested loading capacity was between 50.0 µg and 2.0 mg.

Gradient: For samples pre-cleaned/desalted with C18 SPE or SCX SPE

Time (min)	% mobile phase A	% mobile phase B	%H_2O
0	100	0	0
10	95	5	0
70	65	35	0
85	30	70	0
95	30	70	0
105	100	0	0
120	100	0	0

Concatenation strategy – please see the following reference:

http://www.ncbi.nlm.nih.gov/pubmed/21500348

Reversed-phase chromatography with multiple fraction concatenation strategy for proteome profiling of human MCF10A cells. Wang Y, Yang F, Gritsenko MA, Wang Y, Clauss T, Liu T, Shen Y, Monroe ME, Lopez-Ferrer D, Reno T, Moore RJ, Klemke RL, Camp DG 2nd, Smith RD. **Proteomics**. 2011 May;11(10):2019-26. PMID:21500348