Direct Detect Wet Demo Guideline

Thank you for your interest in Merck Millipore's new Direct Detect IR Spectrometer.

To optimize the outcome of our instrument demonstration in your lab, this information can help you to get the most out of your time:

Please prepare:

- Well calibrated 2 µl pipette with tips (we only need to pipette 2 µl aliquots)
- 2 µl of your sample (with any measurements you have already done on the sample)
- For every buffer used, 2 µl of the buffer your sample is in (for blank)
- With DirectDetect, you do not need to create a new standard curve for every assay – you can save the data on the instrument and refer to it for all subsequent measurements, building up a library of standard curves. If the buffer conditions of your sample differ a lot from those used to create a certain standard curve, it might be necessary to create a new standard curve in the buffer of your choice. (In that case, we would need a very well quantified standard protein with a known concentration, *e.g.* BSA)

Some information on the system:

- Linear dynamic range is 0.2 µg/µl – 5 µg/µl
- Protein quantification is not influenced by reducing agents & detergents (such as DTT, SDS, Tween, etc.)
- Please note that the instrument is not compatible with Urea. As Direct Detect measures the intrinsic amide (= peptide) bond of proteins, measurement is not influenced by the protein sequence. Urea does have a structure almost identical to the peptide bond, obscuring measurements even at concentrations in the mM range.
- Analysis of proteins is independent on the protein structure. Rather, different buffer components that also absorb in the Amide I region of the IR spectrum might influence protein readings. We have several possibilities to get accurate readings of protein concentration even in interfering buffers. Please be prepared to rather test different buffer conditions than different proteins in the same buffer system. If one protein gives a good protein concentration reading on Direct Detect, it is very likely that all proteins will give similar results – in the same buffer conditions.

More info: http://www.millipore.com/life_sciences/flx4/direct_detect