Expressing ^{15}N labeled protein

The following protocol is for 1 Litre of *E.coli* culture. Prior to experiment prepare M9 media *without* NH_4Cl in sterile shaker flasks.

Add to M9 prior to experiment (per litre):

$^{15}NH_4Cl$	1g (can dissolve in 10 mL H2O and filter sterilise into media)
20% glucose	20 mL
vitamins	1 mL
trace elements	10 mL
MgSO4 (1M)	2 mL

Day 1
Transform *E. coli* with desired plasmid.

Day 2:
Early– Use freshly transformed cells to inoculate 5 mL of 2XTY + drug as a pre-culture in rich media to a high OD600.
Late afternoon – Innoculate pre-culture of M9 media (+ $^{15}NH_4Cl$, vitamins, glucose, trace elements, drug) with a 1:100 inoculum of the 2XTY culture. The following day you will need 10 mL of this per 1 litre of media.

Day 3:
Innoculate main culture (supplemented M9 media+ drugs+$^{15}NH_4Cl$) using the overnight M9 culture. (1:100 inoculum, i.e. 10 mL per 1 litre of culture).

At desired OD, set expression temperature and duration as desired and induce as normal. (i.e. 10 mL of 20% arabinose for BAI cells)

Purification:
As normal, however protein needs to be at a final concentration of ~0.5 – 1 mM in 25 mM phosphate buffer, pH 6.5 or lower.
0.1% sodium azide may be added to inhibit bacterial and fungal growth.
A reference compound will be added to the sample such as 0.1 mM DSS
Sample must contain a certain amount of deuterated solvent (usually D2O). (Recommended H2O:D2O ratio is 90:10 or 95:5).
Need to have ~400 - 600 μl of final sample.

Notes on the final sample:
Use buffer components lacking covalently attached protons. The buffer will typically be present at much higher concentrations than that of the biomolecule, thus causing the NMR signals of the buffer to interfere with those of the biomolecule.

Need to add deuterated water (D2O) at a level of 5 % or greater. This is necessary as the stability of the NMR spectrometer is maintained by continuously monitoring the deuterium signal from the solvent.

The total ionic strength should be kept as low as possible (compatible with the solubility/stability of your particular biomolecule). This is important as the coupling of the RF signal in the coil to the sample will be more efficient as the ionic strength is lowered. A salt concentration greater than 100mM typically leads to degradation of spectral quality and spectrometer performance.

The pH should be kept low (below 6.5) if possible. This is because the exchange of the backbone amide proton is base-catalyzed; thus its exchange rate increases as the pH is raised. This can be bad because if the exchange rate becomes too rapid, the amide proton will exchange during the course of the nmr pulse sequence, and therefore the amide group will not be detected.

e.g A buffer of 25 mM dibasic sodium phosphate, 25 mM NaCl, 5% D2O, pH 6.0 should be acceptable.

Reagents

^{15}N Minimal medium (per litre):

1l M9 medium
2ml 1M MgSO4 (2mM)
20ml 20% glucose (0.4%)
1ml vitamins 1000x (see below)
10ml trace elements 100x (see below)
1g $^{15}NH_4Cl$ (can dissolve in 10 mL H_2O and filter sterilise into media)
antibiotic(s)

1l 100x trace elements

in this order:
5g EDTA
0.8g FeCl3
0.05g ZnCl2
0.01g CuCl2
0.01g CoCl2
0.01g H3BO3
1.6g MnCl2
some Ni2SO4
some molybdic acid
dissolve, bring pH to 7.0 with NaOH (some precipitation)
sterile filtered, kept chilled

500ml 1000x vitamins

0.5g riboflavin
0.5g niacinamide
0.5g pyridoxine monohydrate
0.5g thiamine
sterile filtered, kept chilled