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Deuterated recombinant protein production: A high yield, robust and reliable method using Escherichia coli

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In support of neutron scattering studies of proteins, we use a highly reliable method for the deuteration of a broad range of proteins by recombinant expression in Escherichia coli BL21. Typical biomass yields are 40-80 g/L wet weight, yielding 50-500 mg/L purified protein. This method uses a simple, relatively inexpensive defined medium, and routinely results in a high yield expression without need for optimisation. The key elements are: very tight control of expression, careful starter culture adaption steps, media composition and strict maintenance of aerobic conditions ensuring exponential growth. Culture temperature is reduced as required to prevent biological oxygen demand exceeding maximum aeration capacity. The defined medium has glycerol as the sole carbon source and we have not encountered an upper limit for the size of proteins that can be expressed, achieving excellent expression for proteins from 11-154 kDa. The quantity produced at 1L scale ensures that no small angle neutron scattering (SANS), nuclear magnetic resonance (NMR) or neutron crystallography experiment is limited by the amount of deuterated material available. Where difficulties remain, these tend to be cases of altered protein solubility due to high protein concentration and a D2O-based environment. This method is also applied to the recombinant multiple labelling (13C, 15N, 2H) of proteins for NMR investigations.

Keywords

deuteration, triple labelling, protein

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