

¹⁵N, ¹⁵N/¹³C and Randomly D-labelled proteins

Strain usually used : *E. coli* BL21 (DE3)
Growth in M9 minimum media

1st day : transformation

2nd day : precult in LB/antibiotics (or LB 50%D₂O for ¹⁵N/¹³C/D)

9.00 : 1 colonie in 5ml LB/antibiotic

⇩

37°C, 200rpm

18.00 : 400µl of this LB precult are used to inoculate 40ml de labelled M9 ¹⁵N or ¹⁵N/¹³C
(M9 100%D₂O for ¹⁵N/¹³C/D)

⇩

O/N 37°C, 200rpm

3rd day : growth in 1L M9 ¹⁵N or ¹⁵N/¹³C

9.00 : 40ml of the precult are used to inoculate 1L de M9 ¹⁵N (DO₆₀₀=0.05)
(M9 100%D₂O for ¹⁵N/¹³C/D)

⇩

37°C, 200rpm

⇩

When OD₆₀₀ = 0.5 - 0.6 (around 13.00)
induction IPTG (0.5 - 1mM)

⇩

37°C, 200rpm

⇩

the cells are harvested

-after 3hrs induction for M9 ¹⁵N/¹³C (OD_{600 finale}=1, 1.2)

-after 9hrs induction for M9 ¹⁵N/¹³C/D (OD_{600 finale}=1, 1.2)

If this protocol does not work different parameters have to be tested : température, concentration of IPTG, induction time etc.... Even the strain can be changed : BL21 (DE3) plysS or plys E, BL21(DE3) codon+ (stratagene), BL21(DE3)star etc....

MINIMUM MEDIUM M9

Component	Amount (1L)
Na ₂ HPO ₄ ·7H ₂ O	10g
KH ₂ PO ₄	3g
NaCl	0.5g
¹⁵ NH ₄ Cl	1g

⇒ Adjust to pH 7.2 with NaOH before autoclaving

Component (filtrés stérilement)	Amount (1L)
Glucose (¹³ C glucose)	2g (10ml solution 20%)
MgSO ₄	1ml (solution 1M)
CaCl ₂	0.1ml (solution 1M)
Thiamine	2ml (solution 1mg/ml)
Antibiotic	
