

EXPRESSION AND PURIFICATION OF BG4 ANTIBODY

500ng of plasmid is spotted on to 3MM paper within the pencil circle, before putting in a sealable bag. For plasmid recovery, cut the circle out, add 50 μ l of TE, vortex and let rehydrate for 5 min. Quick spin, the supernatant liquid can then be used to transform competent bacteria.

Suggest start with 5 μ l for transformation, using cells that are at least 10^7 in efficiency. 10^8 would be better.

There are no particular problems in growing this plasmid. Routinely the BL21(DE3) strain is used for cloning and preparation of the plasmid. However, as is commonly known, this strain is compromised in transformation efficiency and will produce a lot fewer colonies than standard cloning strains such as XL1-blues or DH5alpha. If there is a problem in transforming BL21s then try a standard high efficiency cloning strain. Once grown up the plasmid can be retransformed into BL21s.

PROTOCOL

Inoculate in 2 ml 2xTY media+2% glucose+50 mg/ml kanamycin
Grow ON at 200 rpm at 30C

Inoculate 200 ml of cell culture in 100 ml auto-induction media
Grow at 37C at 250 rpm for 6 h
Then ON at 25C at 280 rpm

Spin down cell culture for 30' at 4C at 4000g
Resuspend pellet in 8 ml TES, leave 10' on ice
Add 12 ml TES diluted 1:5, leave 15' on ice
Spin down for 10' at 8000g at 4C
Rotate for 1 h at RT with Nickel affinity/anti-his tag beads
Purify on column washing twice with cold PDS+100 mM NaCl+10 mM imidazole, pH 8.0
Elute with PBS+250 mM imidazole, pH 8.0
Dialyze ON in PBS in cold room
Dialyze again OD in PBS in cold room
Store at 4C for few weeks.

BUFFERS

TES buffer

50 mM Tris/HCl pH 8.0
1 mM EDTA pH 8.0
20% sucrose
filter, store at 4C

Auto-induction Medium

Supplement basic ZY medium as follows;

2mM MgSO₄

0.2 x Metals Mix (a small precipitate forms on addition)

1 x 5052

1 x M

50µg/ml kanamycin

1000x metals mix (Stock Solution) - add in order written (per 10ml)

3.6ml distilled MilliQ water

50mM stock FeCl₃.6H₂O (dissolved in ~0.1M HCL) 5ml of a 0.1M

20mM stock CaCl₂ 200 µl of a 1M

10mM stock MnCl₂.4H₂O 100 µl of a 1M

10mM stock ZnSO₄.7H₂O 100 µl of a 1M

2mM stock CoCl₂.6H₂O 100µl of a 0.2M

2mM stock CuCl₂.2H₂O 200µl of a 0.1M

2mM stock NiCl₂.6H₂O 100µl of a 0.2M

2mM stock Na₂MoO₄.2H₂O 200µl of a 0.1M

2mM stock Na₂SeO₃.5H₂O (ppts, then dissolves rapidly) 200µl of a 0.1M

2mM stock 0.1M H₃BO₃ 200µl of a 0.1M

Autoclave the stock solutions of the individual metals (except the FeCl₃ in HCL)

Mix aseptically and store at room temperature

ZY medium (base medium) (per litre)

N-Z amine AS (Sigma Aldrich: N4517) 10g

Yeast Extract 5g

Dissolve in H₂O, autoclave & store at room temperature

1M MgSO₄ (Stock Solution)

Dissolve in H₂O and autoclave.

Store at room temperature.

50x 5052 (Stock Solution)

25% (w/v) glycerol (weigh in beaker)

2.5% (w/v) glucose

10% (w/v) α-lactose

Dissolve in H₂O, autoclave & store at room temperature

50xM (Stock Solution)

1.25M KH₂PO₄

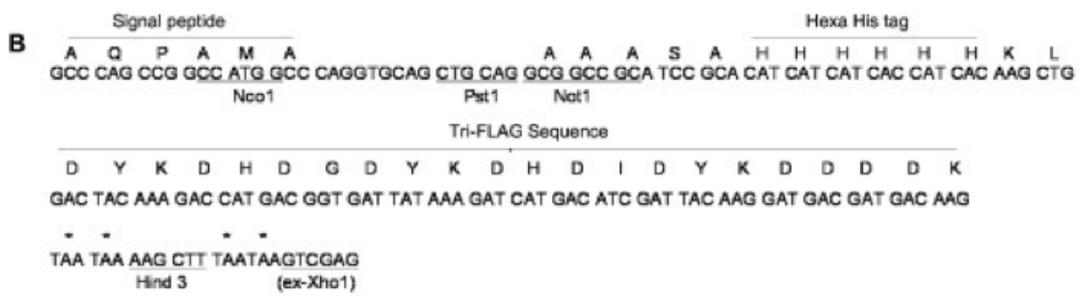
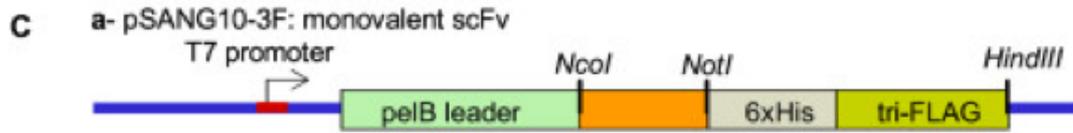
2.5M NH₄Cl

0.25M Na₂SO₄

Dissolve in H₂O, autoclave and store at room temperature.

VECTOR MAP

For details of pSANG10 please see: Martin, C.D., *et al.* (2006). A simple vector system to improve performance and utilisation of recombinant antibodies. BMC Biotechnology 6, 46.



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