

Whole Cell Extract Prep of Gal-Induced Yeast Cells

Inoculate 3 X 5 ml YEP + 2% raffinose with single yeast colony harboring EE-MOT1-expression plasmid. Incubate at 30 degrees O/N.

Next day: check growth of yeast. Cultures may take two days to grow to saturation. Inoculate 1 liter synthetic media + 2% raffinose minus leucine (to select for plasmid) with 10 ml overnight culture at around 5 pm so that starting OD600 is about 0.1. Incubate at 30 degrees O/N with shaking.

Next day: check growth of yeast cells and induce expression by adding 67 ml 30% galactose per liter culture when OD is about 1.0 (probably between 9 am and noon). Incubate for 3-4 hours at 30 degrees with shaking.

Pellet cells at 5000 rpm for 5 minutes in GSA bottles.

Wash cells with 10 ml cold Benoit's Extraction Buffer with BME and protease inhibitors.

Resuspend cells in 10 ml Benoit's buffer and freeze at -80 C or proceed to lyse cells in French Press.

Remove supernatant to eppendorf tubes on ice, spin samples in microfuge for 30 minutes at 4 C. (Alternatively, clarify extract by spinning in ultracentrifuge; see Arindam).

Carefully remove supernatant to new tubes on ice. Save 10 ul in separate tube for protein assay (0.5-5 ul) and western blot (20 µg per lane).

Reagents:

YEP, 1 liter

10 g Bacto Yeast Extract
20 g Bacto Peptone

30% galactose, 30% raffinose

30 g sugar/100 ml final volume
warm in microwave (do not boil), filter sterilize

Synthetic Media minus Leucine, 1 liter

1.2 g Yeast Nitrogen Base (without amino acids and ammonium sulfate)
5.0 g ammonium sulfate
1.0 g amino acid drop out mix (e.g. minus W, U, L)

Add 950 ml water, adjust pH to 7 by adding 0.7 ml 8 M NaOH; autoclave
Before use add 10 ml 100 X uracil and 100X tryptophan (for minus W, U amino acid mix)
Add 5 ml 100 X adenine and stock sugar to 2 % (i.e. 67 ml 30% galactose or raffinose per liter)

Benoit's Extraction Buffer

200 mM TRIS, pH 8.0	3.6 g TRIS
400 mM ammonium sulfate	7.6 g ammonium sulfate
10 mM magnesium chloride	1.5 ml 1 M magnesium chloride
1 mM EDTA	0.6 ml 0.25 M EDTA
10% glycerol	15 ml glycerol
150 ml, adjust pH to 8 with HCl	

add 1 X PMSF, benzamidine, leupeptin, pepstatin, chymostatin and 7mM BME before use

100 X PMSF: 17.4 mg/ml in ethanol; make fresh
100 X Benzamidine: 31 mg/ml in water; make fresh
200 X pepstatin: 0.28 mg/ml in ethanol; store at -20
500 X chymostatin: 1.0 mg/ml in DMSO; store at -20
1000 X leupeptin: 0.3 mg/ml in ethanol; store at -20

stock BME is 14.3 M: dilute in water to 1 M then add appropriately to buffer