Pol III whole cell yeast extract (small scale)

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last modified6/28/99

Grow 2 liters yeast in YPD (with 3% glucose) to an A600 of 1.5 to 2.0 (inoculate ~1.5 ml saturated overnight in 1 liter media and grow ~14 hr at 30 degrees). Expect 5-6 g of cells.

Harvest cells and wash cells once in 30 ml cold H2O.

Wash cells once in 30 ml cold extraction buffer.

Weigh cell pellet.

Scrape pellet into a 10 ml syringe and squirt into a 50 ml screwcap tube which is suspended into liquid N2 (the tube can also contain a little liquid N2 if desired).This cell pellet can be frozen at -70 deg. for at least one year with no loss of activity.

Precool a large (~8 inch diameter) mortar and pestle. Put on dry ice. Add a little liquid N2 to cool.

Dump in frozen yeast and grind big pieces until everything is uniformly small powder (~ 1min).

Add a little more liquid N2 being careful to not make powder fly all over.

Grind again for about 1 min. Repeat liquid N2 addition and 1 min grinding.

Use a precooled spatula to scrape the powder into a precooled weigh boat. Dump into a 50 ml conical tube. Store tube on dry ice if grinding other yeast cells.

After all grinding is complete, transfer tube to ice and add 15% the total volume of extraction buffer (i.e 5 g cells + 750 ul buffer) and let sit on ice 20 min. Scrape the yeast sorbet into an ultraclear SW55 tube and spin in ultracentrifuge 100,000 x g for 1 hr at 4 deg. (33,000 rpm in SW55).

After spinning, ~1/3 volume is cell pellet, ~2/3 volume a clear yellow liquid and a thin white lipid layer on is on top. Remove clear liquid with a syringe being careful to avoid the pellet and lipid layer.

Dialyze 3 X 1 hr at 4 degrees vs 500 ml dialysis buffer.

Aliquot and freeze extracts.

Expect 20-30 mg/ml protein. Typically, use 20 micrograms in a Pol III in vitro transcription reaction.

Solutions needed for WCE:

Extraction Buffer (500 ml)

100 mM HEPES, 7.9 11.9 g HEPES
245 mM KCl 9.14 g KCl
5 mM EGTA 10 ml 0.25 M EGTA
1 mM EDTA 2 ml 0.25 M EDTA
H20 to 500 ml
Just before use, add protease inhibitors and add DTT to 2 mM.

Dialysis Buffer 1.5 liters:

20 mM HEPES, 7.9 7.14 g HEPES
100 mM KCl 11.2 g KCl
5 mM MgCl2 7.5 ml 1 M MgCl2
1 mM EDTA 6 ml 0.25 M EDTA
20% glycerol 300 ml glycerol
H20 to 1.5 liter
Just before use, add protease inhibitors and add DTT to 2 mM.

Protease inhibitors and DTT:

0.1 M PMSF (100x)
16 mg/ml Ethanol
Store at -20 degrees

0.2M DTT
32 mg/ml H2O
Store frozen at -20 degrees

Benzamidine (100X)

31 mg/ml H2O.
Store frozen at -20 degrees

Leupeptin (500X)
0.15 mg/ml Ethanol.
Store at -70 degrees for less than 6 months

Pepstatin (200X)
0.28 mg/ml methanol
Store at -20 degrees.

Chymostatin (2,500X)
5mg/ml DMSO
Store frozen at -20 degrees