Glucose-Limited Medium

Nikolai Slavov

1 ORIGIN

This protocol describes the composition of the growth medium used by Brauer *et al* (2008); Slavov and Botstein (2013) for studying the growth rate response and by Slavov and Botstein (2011, 2010); Slavov *et al* (2012) to synchronize metabolically continuous cultures of budding yeast. It is based on the composition of the medium described by Saldanha *et al* (2004) and later in the protocols of the Dunham and Botstein labs. We have demonstrated that in this medium the growth of budding yeast is limited on glucose and both the final biomass of batch cultures and the steady–state biomass density of continuous cultures depend linearly on the concentration of glucose.

2 COMPOSITION OF THE MEDIUM

Chemical	Amount
$10\times$ Salts, see Table 2	1.0 L
$1000\times$ metals, see Table 3	10.0 mL
$1000\times$ vitamins, see Table 4	10.0 mL
Glucose	8 g[1]
MilliQ Water	fill up to 10L

Table 1. Composition of the medium.

[1]The amount of glucose may vary when explicitly specified, i.e (Slavov and Botstein, 2011). We have verified that up to $3.4g/L$ ($34g$ of glucose in the protocol in Table 1), the medium remains glucose-limited.

2.1 SALTS

Salts are made as a $10\times$ stock that keeps at room temperature for at least a year, see Table 2. Make the salts in sterile glass with distilled water and autoclave. Be vigilant about shaking before using.

Amount	Mineral Salt	Storage
5 g	$CaCl_2 2H_2O$	RT shelf
5 g	$NaCl$	RT shelf
25 g	$MgSO_4 7H_2O$	RT shelf
50 g	KH_2PO_4	RT shelf
250 g	$(NH_4)_2SO_4$	RT shelf
fill up to 5L	MilliQ Water	Filtration System

Table 2. Mineral salts used for making $10\times$ stock solution of the salts.

2.2 METALS

Metals are made as a $1000\times$ stock that keeps at room temperature for at least a year, see Table 3. Keep the bottle well wrapped in foil since some of the metals are light sensitive. Make the metals in sterile glass with distilled water. Be vigilant about shaking before using since the metals will not totally dissolve. Dissolve the mineral salts in Table 3 in $900mL$ distilled water, in stirring glass.

Amount	Metal Salt	Storage
500 mg	boric acid	RT shelf
40 mg	copper sulfate.5H2O	RT shelf
100 mg	potassium iodide	RT, dark, dessicator
200 mg	ferric chloride.6H2O	RT shelf
400 mg	manganese sulfate.H2O	RT shelf
200 mg	sodium molybdate.2H2O	RT shelf
400 mg	zinc sulfate.7H2O	RT shelf

Table 3. Mineral salts used for making $1000\times$ stock solution of the metals.

Bring total volume to $1L$ with MilliQ water, and pour into a bottle. Cover the bottle with foil, and store at room temperature.

2.3 VITAMINS

Vitamins are also made as a $1000\times$ stock, see Table 4. The solution is aliquoted into $50ml$ Falcon tubes and stored at -20^oC. Don not fill the tubes to the top, or else the lid will split when frozen. The "working tube" can be stored at 4^oC. The vitamins will not dissolve completely, so shake before use. Care should be taken to keep the solution well mixed while aliquoting. Weight all chemicals and add to a beaker of stirring glass distilled water to dissolve as much as possible. Top off to $1L$, then aliquot about $40mL$ per $50mL$ tube, and freeze.

Amount	Vitamin	Storage
2 mg	biotin	4^oC
400 mg	calcium pantothenate	4^oC
2 mg	folic acid	RT, dark, dessicator
2000 mg	inositol (aka myo-inositol)	RT shelf
400 mg	niacin (aka nicotinic acid)	RT shelf
200 mg	p-aminobenzoic acid	4^oC
400 mg	pyridoxine HCl	RT, dark, dessicator
200 mg	riboflavin	RT shelf
400 mg	thiamine HCl	RT, dark, dessicator

Table 4. Vitamins used for making $1000\times$ stock solution of the vitamins

References

Brauer MJ, Huttenhower C, Airoldi EM, Rosenstein R, Matese JC, Gresham D, Boer VM, Troyanskaya OG, Botstein D (2008) Coordination of Growth Rate, Cell Cycle, Stress Response, and Metabolic Activity in Yeast. *Mol Biol Cell* **19**: 352–367

Saldanha AJ, Brauer MJ, Botstein D (2004) Nutritional Homeostasis in Batch and Steady-State Culture of Yeast. *Mol Biol Cell* **15**: 4089–4104

Slavov N, Airoldi EM, van Oudenaarden A, Botstein D (2012) A conserved cell growth cycle can account for the environmental stress responses of divergent eukaryotes. *Molecular Biology of the Cell* **23**: 1986–1997

Slavov N, Botstein D (2010) *Universality, specificity and regulation of S. cerevisiae growth rate response in different carbon sources and nutrient limitations*. Ph.D. thesis, Princeton University

Slavov N, Botstein D (2011) Coupling among growth rate response, metabolic cycle, and cell division cycle in yeast. *Mol Biol Cell* **22**: 1997–2009

Slavov N, Botstein D (2013) Decoupling Nutrient Signaling from Growth Rate Causes Aerobic Glycolysis and Deregulation of Cell-Size and Gene Expression. *Molecular Biology of the Cell* **24**: 157–168