

Materials List for:

Ratiometric Biosensors that Measure Mitochondrial Redox State and ATP in Living Yeast Cells

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Materials

Name	Company	Catalog Number	Comments
			Reagents
Antimycin A	Sigma-Aldrich (St. Louis, MO)	1397-94-0	Dissolved in ethanol to a 2 mg/ml stock solution.
SGlyc (synthetic glycerol-based) yeast growth medium *omit for SGlyc-Ura **omit for SGlyc-Leu			Dissolve in H ₂ O. Adjust pH to 5.5 with NaHCO ₃ . Autoclave. <i>Ingredients:</i> 0.67% Yeast nitrogen base without amino acids 3% Glycerol 0.05% Glucose 2 mg/ml adenine 2 mg/ml uracil* 1 mg/ml L-arginine 1 mg/ml L-histidine 1 mg/ml L-leucine** 3 mg/ml L-lysine 2 mg/ml L-methionine 4 mg/ml L-phenylalanine 2 mg/ml L-tryptophan 3 mg/ml L-tyrosine
SC (synthetic complete, glucose-based) yeast growth medium *omit for SGlyc-Ura **omit for SGlyc-Leu			Dissolve in H ₂ O. Adjust pH to 5.5 with NaHCO ₃ . Autoclave. <i>Ingredients:</i> 0.67% Yeast nitrogen base without amino acids 3% Glucose 2 mg/ml adenine 2 mg/ml uracil* 1 mg/ml L-arginine 1 mg/ml L-histidine 1 mg/ml L-leucine** 3 mg/ml L-lysine 2 mg/ml L-methionine 4 mg/ml L-phenylalanine 2 mg/ml L-tryptophan 3 mg/ml L-tyrosine
Valap			Combine ingredients in a 1:1:1 (w:w:w) ratio. Melt by submerging in a 70 °C H ₂ O bath. Aliquot into glass petri dishes. Store at room temperature. <i>Ingredients:</i> Vaseline petroleum jelly, hard paraffin, lanolin
			Equipment and Software

Precleaned Gold Seal Rite-on Micro Slides	Thomas Scientific (Swedesboro, NJ)	3050	Size: 25 x 75 mm; Thickness: 0.93 to 1.05 mm
High-performance coverslips, No. 1.5, 18x18 mm	Zeiss (Thornwood, NY)	474030-9000-000	These are less variable in thickness ($170\pm 5\ \mu\text{m}$) than standard coverslips, reducing spherical aberration and improving 3D imaging performance
Fisherbrand Microscope Cover Glass, No. 1.5	Fisher Scientific (Pittsburgh, PA)	12-545E	Size: 22 x 22 mm, No. 1.5 thickness ($170\ \mu\text{m}$)
A1 laser scanning confocal microscope with spectral detector and 100x/1.49 NA Apo-TIRF objective	Nikon (Melville, NY)		
AxioObserver.Z1 microscope equipped with a 100x/1.3NA EC Plan-Neofluar objective (Zeiss) and Orca ER cooled CCD camera (Hamamatsu) and controlled by Axiovision software	Zeiss (Thornwood, NY); Hamamatsu (Hamamatsu City, Japan)		
Volocity 3D Image Analysis software	Perkin Elmer (Waltham, MA)		Restoration module for deconvolution; Quantitation module for ratio calculation and measurement
ImageJ software	National Institutes of Health (Bethesda, MD)		http://rsb.info.nih.gov/ij/