

Materials List for:

Biochemical and High Throughput Microscopic Assessment of Fat Mass in *Caenorhabditis Elegans*

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Materials

Name	Company	Catalog Number	Comments
96-pin replicator	Nunc	250520	
LB Agar	US Biologicals	L1500	
Agarose	Invitrogen	16500500	
Mechanical Pipettors	Biohit M Line	M10, M100, M300	
Electronic Pipettor	Biohit E Line	E1200	
1 M KH ₂ PO ₄	Sigma	P0662	pH 6.0 Sterilized by Autoclaving
1 M MgSO ₄	Sigma	M2643	Sterilized by Autoclaving
1 M CaCl ₂	Sigma	C2661	Sterilized by Autoclaving
NaCl	Sigma	S5886	
5 mg/ml Cholesterol	Sigma	C8667	In 100% Ethanol
200 mg/ml Carbenicillin	US Biologicals	C1100	Filter Sterilized 0.2 µ
1 M IPTG	US Biologicals	I8500	Filter Sterilized 0.2 µ
100 mg/ml Ampicillin			Filter Sterilized 0.2 µ
5 mg/ml Tetracycline			In 100% Ethanol
Bacto Agar	BD	214040	
96-well TC Plates	BD-Falcon	353072	For 96-well RNAi
Rectangular A/T Plates	Thermo Scientific	242811	For stamping RNAi
LB Media	US Biologicals	L1530	Prepared as per manufacturer instructions
96-well Deep Well Assay Blocks	Corning	Costar 3960	Rectangular V-bottom assay block
96-well Shallow Well Assay Block	Corning	Costar 3956	Round V-bottom assay block
Sterile Sealing Film	VWR	89134-432	
Aluminum Sealing Film	Corning	Costar 6570	Thermowell Sealing tape for 96-well plates
M9 Buffer	See Hope <i>et al.</i> ²⁰		See recipe below
Triton X-100	Bio-Rad	161-0407	
Nile red	Invitrogen	N-1142	
Isopropanol	Fisher	BP2632-4	
96-pin aspirator	VP Scientific	VP177A-1	
96-well Teflon Masked Microscope Slides	Thermo Scientific	Custom	Can also order Trevigen 96-well Cometslide
96-well Gold-Seal Coverslides	Thermo Scientific	Custom	Can also use second 96-well Cometslide
Deep-well PCR plate	VWR	89049-178	

Non-sterile adhesive film	VWR	60941-070	
High-Throughput Microscope	Leica	DM6000 fully automated with Prior 96-well stage	With Chroma 49002 GFP filter set and 2x objective
Bath Sonicator	Diagenode	Bioruptor XL	
Chloroform	Sigma	366927-4L	
Methanol	Fisher	Optima F456-4	
Phospholipid Standard	Avanti Polar Lipids	830456P	1,2-diheptadecanoyl- <i>sn</i> -glycero-3-phospho-(1'- <i>rac</i> -glycerol)
Triglyceride Standard	NuChek Prep	T-135	tritridecanoin
Wiretrol 50 µl Pipet	Fisher	21-176-3A	Drummond Scientific
Acetone	Sigma	320110-4L	
Sulfuric Acid	Fisher	A300-500	
Hexane	Fisher	Optima H306-1	
SPE Silica Column	Thermo Scientific	60108-317	Hypersep SI, 100 mg resin bed
Solid Phase Chromatography Manifold	Sigma	57265	Supelco Visiprep 24-DL
Borosilicate Pipets	Fisher	13-678-27F	10 ml size
Borosilicate Pasteur Pipets	Fisher	13-678-8B	
Borosilicate Culture Tubes	Fisher	14-961-27	
Threaded Borosilicate Tubes	VWR	14235-460	8 ml capacity
Phenolic Caps for Culture Tubes	Fisher	14-823-211	
GC/MS Autosampler Vials	Agilent	5188-6592	
Caps for Autosampler Vials	Agilent	5185-5861	
GC/MS	Agilent	6890 GC / 5973 MSD	Outfitted with a Supelcowax-10 column

Nematode Growth Media (NGM): for 25 96-well RNAi plates, prepare 1 L of NGM:

Reagent	Amount
Deionized water	1 L
NaCl	3 g
Agarose	17 g
Bacto peptone	2.5 g
Autoclave liquid cycle 121 °C for 40 min with a stir bar. Allow to cool to 60 °C stirring on a hot plate set at 60 °C. Then add the following:	
1 M MgSO ₄	1 ml
5 mg/ml cholesterol	1 ml
1 M KH ₂ PO ₄ (pH 6.0)	25 ml
1 M CaCl ₂	1 ml
200 mg/ml carbenicillin	1 ml
1 M IPTG	5 ml
Dispense into 96-well RNAi plates. Store at 4 °C until use, up to 2 weeks.	

Rectangular Ampicillin/Tetracycline LB Agar Plates (A/T): for 20 A/T plates, prepare 1 L of media:

Reagent	Amount
Deionized water	1 L
LB agar (US Biologicals)	32 g
2 M NaOH	1.5 ml
Bacto agar	2.5 g
Autoclave liquid cycle 121 °C for 40 min with a stir bar. Allow to cool to 60 °C stirring on a hot plate set at 60 °C. Then add the following:	

Tetracycline	3 ml
100 mg/ml Ampicillin	0.5 ml
Dispense 45 ml into each plate. Store in dark at 4 °C until use, up to 4 weeks.	

LB bacterial growth media: for 4 L, sufficient for growing and resuspending 25 96-well blocks

Mix 62 g LB powder (US Biologicals), 4 L distilled water, and 4.8 ml 2 M NaOH. Autoclave in 1 L or 0.5 L aliquots, liquid cycle, 40 min. Can be stored up to 1 year in airtight bottles at room temperature. Discard if cloudy.

100 mg/ml Ampicillin

Mix 5 g ampicillin powder and deionized water to a total volume of 50 ml. Filter sterilize 0.2 µ, dispense in 1 ml aliquots and freeze at -20 °C until use.

5 mg/ml Tetracycline

Add 1.25 g tetracycline powder to 250 ml 100% ethanol. Mix well until fully dissolved and store at -20 °C in a light-tight container.

200 mg/ml Carbenicillin

Mix 10 g carbenicillin powder and deionized water to a total volume of 50 ml. Filter sterilize 0.2 µ, dispense in 1 ml aliquots and freeze at -20 °C until use.

5 mg/ml Cholesterol

Add 1.25 g cholesterol to 250 ml 100% ethanol. Mix well and store at 4 °C for up to 6 months.

1 M MgSO₄

Dissolve 120.37 g MgSO₄ in 800 ml deionized water. Bring final volume to 1 L. Autoclave 40 min liquid cycle and store at room temperature.

1 M CaCl₂

Dissolve 110.98 g CaCl₂ in 800 ml deionized water. Bring final volume to 1 L. Autoclave 40 min liquid cycle and store at room temperature.

1 M KH₂PO₄, pH 6.0

Dissolve 136.09 g KH₂PO₄ in 800 ml deionized water. pH to 6.0 and bring final volume to 1 L. Autoclave 40 min liquid cycle and store at room temperature.

M9 Buffer: for 1 L—we routinely make 8-10 L batches

Dissolve 3 g KH₂PO₄, 6 g Na₂HPO₄, 5 g NaCl, 1 ml 1 M MgSO₄ in 900 ml. Bring final volume to 1 L. Aliquot in 100-500 ml bottles and autoclave 40 min on liquid cycle and store at room temperature.

10 N NaOH

Dissolve 400 g NaOH in 800 ml deionized water, allow to cool and bring to a final volume of 1 L. Store in 50 ml tubes at room temperature.

1 M IPTG

Add 23.81 g IPTG to deionized water and bring total volume to 100 ml. Filter sterilize 0.2 µ, dispense in 1 ml aliquots and freeze at -20 °C until use.

Egg Preparation Bleach/NaOH Solution: 20 ml sufficient for one egg preparation

Add 4 ml bleach, 1 ml 10 N NaOH, 5 ml H₂O, and 10 ml M9 buffer to a 50 ml polypropylene conical tube. Make immediately before use.

Nile red stock solution

Add 50 ml 100% Acetone to 25 mg Nile red powder. Mix well and store at room temperature in light-tight parafilm container. Can be stored for months in this manner.

Nile red staining solution: for 500 ml, sufficient for staining 30 x 96-well plates

Add 3 ml Nile red stock solution to 500 ml 40% v/v isopropanol. Store at room temperature in foil-wrapped glass bottle. Prepare immediately before use.

Phospholipid Standard

Weigh 7.6 mg phospholipid standard, 1,2-diheptadecanoyl-*sn*-glycero-3-phospho-(1'-*rac*-glycerol), in a glass beaker. Dissolve in 20 ml 2:1 chloroform:methanol (25 nanomoles per 50 µl). Keep covered with aluminum foil until use. Standard can also be kept at -80 °C in an airtight glass vial with phenolic cap for 1 year.

Triglyceride Standard

Weigh 6.8 mg triglyceride standard, tritridecanoin, into a glass beaker. Dissolve in 30 ml 2:1 chloroform:methanol (16.7 nanomoles per 50 μ l). Keep covered with aluminum foil until use. Standard can also be kept at -80 °C in an airtight glass vial with phenolic cap for 1 year.