Department of Forensic Biology

Solutions Manual

Version 1.0

April 2, 1992
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I

Forensic Biochemistry & Hematology Laboratory

Solutions Manual

Version 1.0

April 2, 1992
ACETIC ACID ANODE SOLUTION

standard batch size: 250ml

Reagents

glacial acetic acid

Procedure

1. Mix 2.5ml glacial acetic acid with 247.5ml distilled water.
2. Store at room temperature.
ACID PHOSPHATASE REACTION BUFFER

standard batch size: 200ml

Reagents

citric acid

sodium hydroxide

Procedure

1. Dissolve 1.92g citric acid and 0.80g sodium hydroxide in 2L distilled water.

2. Adjust the pH to 5.0, if necessary, by adding additional sodium hydroxide.

3. Store at 2-5°C.
ACID PHOSPHATASE SPOT TEST REAGENT

standard batch size: variable

Reagents

commercial spot test reagent

OR

sodium alpha-naphthyl phosphate

fast blue B salt

anhydrous sodium acetate

Procedure

1. Aliquot 1.58g portions of commercially prepared acid phosphatase spot test reagent and store in microcentrifuge tubes at freezer temperatures.

2. For use, dissolve an aliquot in 5ml distilled water.

OR

1. Dissolve 5mg sodium alpha-naphthyl phosphate and 5mg fast blue B salt separately in 5ml of buffer (prepared by dissolving 8.21g anhydrous sodium acetate in 1L distilled water and adjusting to pH 5.5 with acetic acid).
ALKALINE SUBSTRATE BUFFER

Standard batch size: 1 L

Reagents

diethanol amine
sodium azide
MgCl₂
HCl

Procedure

1. Dissolve 97 mL diethanolamine, 0.2 g sodium azide, and 0.1 g MgCl₂ in 800 mL distilled water.
2. Adjust to pH 9.8 with HCl.
3. Make up to 1 L with distilled water.
4. Store at 2-5°C.

April 22, 1993
ALSEVIER’S BUFFER

standard batch size: 500ml

Reagents

trisodium citrate, dihydrate
citric acid, anhydrous
dextrose
sodium chloride

Procedure

1. Dissolve 4.0g trisodium citrate dihydrate, 0.25g anhydrous citric acid, 10.25g dextrose, and 2.09g sodium chloride in 500ml distilled water.

2. Adjust to pH 6.0, if necessary, using either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3. Store at 2-5°C.
AMYLASE GEL BUFFER

standard batch size: 1L

Reagents

anhydrous sodium phosphate, monobasic
anhydrous sodium phosphate, dibasic
sodium chloride

Procedure

1. Dissolve 5.4g anhydrous sodium phosphate, monobasic, 7.8g anhydrous sodium phosphate, dibasic, and 0.4g sodium chloride in 1L distilled water.

2. Adjust to pH 6.9, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3. Store at 2-5°C.
ANTI-H LECTIN

standard batch size: variable

Reagents

Ulex europaeus seeds

saline

Procedure

1. Grind 10.0g Ulex europaeus seeds (or multiple thereof) in a grinder or blender. If a grinder or a blender is not available, a mortar and pestle can be used.

2. Soak the ground seed in saline (50ml/10g seeds) for 48-72 hours at 2-5°C.

3. Centrifuge and discard seeds and other solids.

4. Filter supernatant using gentle suction if necessary.

5. Incubate supernatant at 60°C for 30 minutes.

6. Centrifuge and discard solid material.

7. Store supernatant in sterilized glass dropper bottles at 2-5°C.
CASEIN STOCK SOLUTION

Standard batch size: 1 L

Reagents

Hammerstein casein
sodium azide
phosphate buffered saline
NaOH

Procedure

1. Thoroughly dissolve 10 g Hammerstein casein in 500 mL distilled water. The casein is very slow to go into solution.
2. Adjust to pH 8.0 with NaOH.
3. Add 500 mL PBS and 0.1 g sodium azide.
4. Store frozen in 40 mL aliquots.
Initials: ECJ Date: 4/2/92

COOMASSIE BLUE STAIN

standard batch size: 1L

Reagents

brilliant blue R
methanol
glacial acetic acid
distilled water

Procedure

1. Mix together 500ml methanol, 100ml glacial acetic acid, and 400ml distilled water.

2. Add 1.0g brilliant blue R to the solution and stir for several minutes.

3. Filter the solution directly into a storage bottle.

4. Store at room temperature.
CRUDE PANCREATIC EXTRACT

standard batch size: variable

Reagents

human pancreatic tissue

sodium acetate

calcium chloride

Procedure

1. Homogenize human pancreatic tissue in 0.1M sodium acetate containing 1mM calcium chloride at a concentration of 20g/L and a pH of 6.5.

2. Centrifuge the homogenate and pipette 100ul aliquots of the resulting supernatant into microcentrifuge tubes and freeze.
CRUDE SALIVARY EXTRACT

standard batch size: variable

Reagents

human saliva

calcium chloride

Procedure

1. Pool saliva from several individuals and centrifuge.

2. Add calcium chloride to the supernatant to a final concentration of 1mM.

3. Divide the supernatant into 100ul aliquots, place in microcentrifuge tubes and freeze.
DESTAIN SOLUTION

standard batch size: 4L

Reagents
methanol
glacial acetic acid
distilled water

Procedure

1. Mix together 1816ml methanol, 1816ml distilled water, and 364ml glacial acetic acid.

2. Transfer to a 4L storage bottle and keep at room temperature.
DITHIOREITOL (DTT)

standard batch size: variable

Reagents

dithiothreitol

Procedure

1. Dissolve 0.31g DTT in 40ml distilled water.

2. Dispense approximately 1ml aliquots of DTT solution into microcentrifuge tubes.

3. Store at freezer temperatures.
Initials: RG Date: 4/1/92

ESD REACTION BUFFER

standard batch size: 2L

Reagents

sodium acetate, anhydrous

Procedure

1. Dissolve 8.2g anhydrous sodium acetate in 2L distilled water.
2. Adjust the pH to 6.5, if necessary, using 1% acetic acid.
3. Store at 2-5°C.
ESD/PGM GEL BUFFER

standard batch size: 2L

Reagents

ESD/PGM tank buffer

Procedure

1. Mix 133ml ESD/PGM tank buffer with 1867ml distilled water.
2. Store at 2-5°C.
ESD/PGM TANK BUFFER

standard batch size: 18L

Reagents

tris base
maleic acid
EDTA free acid
magnesium chloride, hexahydrate
sodium hydroxide

Procedure

1. Dissolve 218.0g tris base, 209.2g maleic acid, 52.6g EDTA free acid, 36.5g magnesium chloride, hexahydrate, and 90.0g sodium hydroxide in 2-4L distilled water.

2. Transfer solution to 20L carboy and bring to a final volume of 18L with distilled water.

3. Adjust the pH to 7.4, if necessary, by using either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

4. Store at 2-5°C.
ETHANOLAMINE CATHODE SOLUTION

standard batch size: 250ml

Reagents

ethanolamine

Procedure

1. Mix 2.5ml ethanolamine with 247.5ml distilled water.
2. Store at room temperature.
FICIN 4%

standard batch size: variable

Reagents

ficin

Alsevier's buffer

Procedure

1. Dissolve 1.0g ficin in 25.0ml Alsever's buffer.

2. Filter the solution through Whatman #1 filter paper using suction, if necessary.

3. Dispense 200ul aliquots of the filtered ficin solution into microcentrifuge tubes.

4. Store aliquots at freezer temperatures.
GC GEL BUFFER

standard batch size: 2L

Reagents

anhydrous sodium phosphate, dibasic

citric acid, anhydrous

Procedure

1. Dissolve 1.62g anhydrous sodium phosphate, dibasic and 0.96g anhydrous citric acid in 2L distilled water.

2. Adjust the pH to 5.5, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3. Store at 2-5°C.
GC TANK BUFFER

standard batch size: 18L

Reagents

anhydrous sodium phosphate, dibasic
citric acid, anhydrous

Procedure

1. Dissolve 741.6g anhydrous sodium phosphate, dibasic and 345.6g anhydrous citric acid in 2-4L distilled water.

2. Transfer solution to 20L carboy and bring to a volume of 18L with distilled water.

3. Adjust to pH 5.5, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

4. Store at 2-5°C.
IEF POLYACRYLAMIDE PLATES

standard batch size: variable

Reagents

See tables 1 and 2.

Procedure

1. Using table 1 for appropriate quantities of reagents, add the sucrose, acrylamide premix (or equivalent), and riboflavin (or ammonium persulfate) to distilled water and dissolve by gentle agitation.

2. Once solution is clear, add the appropriate type and quantity of ampholyte(s) (see table 2). For PGM subtype plates, EPPS/HEPPS is added and dissolved by gentle agitation.

3. The gel solution is then casted on glass plates and allowed to polymerize (3-3.5ml/10x20cm plate, 4-4.5ml/13x20cm plate, 6-6.5ml/15x20cm plate, 8-8.5ml/13x27cm plate).

Table 1

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Number of Plates Required</th>
<th>Units</th>
</tr>
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<tbody>
<tr>
<td>Distilled water</td>
<td>5-6  10-12  15-18  20-24</td>
<td>ml</td>
</tr>
<tr>
<td>Sucrose</td>
<td>20  40  60  80</td>
<td>g</td>
</tr>
<tr>
<td>3% Acrylamide Premix OR</td>
<td>1.0  2.0  3.0  4.0</td>
<td>g</td>
</tr>
<tr>
<td>5% Acrylamide Premix OR</td>
<td>0.6  1.2  1.8  2.4</td>
<td>g</td>
</tr>
<tr>
<td>Acrylamide OR</td>
<td>0.4  0.8  1.2  1.6</td>
<td>g</td>
</tr>
<tr>
<td>Acrylamide</td>
<td>0.97 1.94 2.91 3.88</td>
<td>g</td>
</tr>
<tr>
<td>Bisacrylamide</td>
<td>0.03 0.06 0.09 0.12</td>
<td>g</td>
</tr>
<tr>
<td>Riboflavin (1.0mg/10mlH₂O) OR</td>
<td>150 300 450 600</td>
<td>ul</td>
</tr>
<tr>
<td>Ammonium Persulfate (0.23g/5mlH₂O)</td>
<td>150 300 450 600</td>
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Table 2

<table>
<thead>
<tr>
<th>System</th>
<th>Ampholyte(s)</th>
<th>Number of Plates Required</th>
<th>Units</th>
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</thead>
<tbody>
<tr>
<td>ACP</td>
<td>pH 4-8</td>
<td>5-6 1.0 10-12 2.0 15-18 3.0 20-24 4.0</td>
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<tr>
<td>OR</td>
<td>pH 4-6</td>
<td>0.5 1.0 1.5 2.0</td>
<td>ml</td>
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<tr>
<td>OR</td>
<td>pH 6-8</td>
<td>0.5 1.0 1.5 2.0</td>
<td>ml</td>
</tr>
<tr>
<td>ESD</td>
<td>pH 4-6.5</td>
<td>1.0 2.0 3.0 4.0</td>
<td>ml</td>
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<td>PGM</td>
<td>pH 5-7</td>
<td>1.0 2.0 3.0 4.0</td>
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<td>EPPS/HEPPS</td>
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<td>Hb</td>
<td>pH 6-8</td>
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<td>Hb</td>
<td>pH 7-9</td>
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<tr>
<td>Hb</td>
<td>pH 3-10</td>
<td>0.2 0.4 1.5 2.0</td>
<td>ml</td>
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</table>
IODINE SOLUTION

standard batch size: 1L

Reagents

potassium iodine
iodine

Procedure

1. Dissolve 16.5g potassium iodine and 25.4g iodine in 1L warm distilled water.

2. Stir for 15 minutes and then filter using suction, if necessary.

3. Store in a brown glass bottle at 4°C.
KIDNEY BEAN EXTRACT (KBE)

standard batch size: variable

Reagents

red kidney beans
saline

Procedure

1. Shell commercially purchased red kidney beans and powder in a blender.

2. Soak powder in physiological saline at 4°C at a concentration of 25g/L.

3. Centrifuge the mixture and pipette 100ul aliquots of the resulting supernatant in microcentrifuge tubes and freeze.
LEUCOMALACHITE GREEN SOLUTION

standard batch size: 250 ml

Reagents

leucomalachite green (oxalate salt)
glacial acetic acid
zinc dust

Procedure

1. Mix together 1g leucomalachite green, 100ml glacial acetic acid, 150ml distilled water, and 5g zinc dust.

2. Reflux until solution is a clear light yellow color. This may take several hours.

3. Allow to cool and then filter.

4. Store in a dark glass bottle at 4°C over additional zinc dust.

CAUTION: Hydrogen gas is generated. Do not seal bottle tightly.
P30 AGAROSE GELS

standard batch size: variable

Reagents

P30 tank buffer

Sigma type III agarose
(or equivalent)

Procedure

1. Dissolve 3.0g Sigma type III agarose (or equivalent) in 300ml P30 tank buffer by heating to a boil on a stir plate.

2. Once solution is clear, dispense 7ml aliquots into 20x150mm test tubes.

3. Allow gels to solidify, then cover tubes with parafilm and store at 2-5°C.
P30 TANK BUFFER

standard batch size: 8L

Reagents

tris base
EDTA free acid
boric acid

Procedure

1. Dissolve 201.6g tris base, 20.0g EDTA free acid, and 15.2g boric acid in 2-4L distilled water.

2. When the solution is clear, place it in a carboy and adjust the volume to 8L with distilled water.

3. Adjust to pH 9.1, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

4. Store at 2-5°C.
PGM REACTION BUFFER

standard batch size: 2L

Reagents

tris base

magnesium chloride, hexahydrate

Procedure

1. Dissolve 24g tris base and 8.0g magnesium chloride, hexahydrate in 2L distilled water.

2. Adjust the pH to 8.0, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3. Store at 2-5°C.
PGM REACTION MIXTURE

standard batch size: variable

Reagents

- glucose 1-phosphate with 1% glucose 1,6-diphosphate
- NADP sodium salt
- MTT

Procedure

1. Grind together 3.5g glucose 1-phosphate with 1% glucose 1,6-diphosphate, 0.2g NADP sodium salt, and 0.3g MTT forming a homogeneous powder. The open end of a test tube can be used to grind the powder in a beaker.

2. Equally divide the mixture into approximately 70-75 portions and place aliquots in plastic microcentrifuge tubes.

3. Store at freezer temperatures.
PHENOLPHTHALIN SOLUTION

standard batch size: 1L

Reagents

phenolphthalin

potassium hydroxide

Procedure

1. Dissolve 2.0g phenolphthalin in 200mL distilled water forming a dark pink solution.

2. Add 10.0g potassium hydroxide to the pink solution. Stir until the solution is clear.

3. Mix this 200ml solution with 800ml ethanol.

4. Transfer solution to a dark glass bottle and add enough zinc dust to cover the bottom.

5. The solution should be sealed tightly and stored at 2-5°C.
Initials: JCS  Date: 11/14/93

PHYSIOLOGICAL SALINE

standard batch size: 10 Liters

Reagents

sodium chloride

Procedure

1. Dissolve 88.8g of sodium chloride in 10 liters of distilled water.

2. Store at 4°C.
**PHOSPHATE BUFFERED SALINE (PBS)**

standard batch size: 1L

**Reagents**

monohydrate sodium phosphate, monobasic
heptahydrate sodium phosphate, dibasic
sodium chloride

**Procedure**

1. Dissolve 5.38g monohydrate sodium phosphate, monobasic, 16.35g heptahydrate sodium phosphate, dibasic and 9.0g sodium chloride in 1L distilled water.

2. Adjust to pH 7.0, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).

3. Store at 2-5°C.
PHOSPHATE BUFFERED SALINE (PBS)
(from pre–made concentrated tablets)
Standard batch sizes: 200 mL, 1 L

**Reagents**
PBS tablets

**Procedure**
1. To prepare 200 mL, dissolve 1 tablet in 200 mL of distilled water.
2. To prepare 1 L, dissolve 5 tablets in 1 L distilled water.
3. Store at 2–5°C.
PBS w/ 0.1% bovine serum albumin (PBS-BSA)

Standard batch size: 100 mL

Reagents

bovine serum albumin

phosphate buffered saline

Procedure

1. Add 100 uL bovine serum albumin to 100 mL of PBS.

2. Use immediately to prepare stock solution of P30 antigen or store at 2-5°C.
PBS w/ 0.02% w/v CASEIN (PBS–Casein)

Standard batch size: 2 L

Reagents

casein stock solution

phosphate buffered saline

Procedure

1. Add 40 mL casein stock solution to 2 L of PBS.

2. Store at 2–5°C.
POTASSIUM CYANIDE SOLUTION 0.05%
standard batch size: 200ml

Reagents
potassium cyanide

Procedure
1. Dissolve 0.1g potassium cyanide in 200ml distilled water.
2. Store at room temperature.
SPECIES AGAROSE GELS

standard batch size: variable

Reagents

species tank buffer

Sigma type I agarose
(or equivalent)

Procedure

1. Mix 150ml species tank buffer with 150ml distilled water.

2. Dissolve 3g of Sigma type I agarose (or equivalent) in the solution by heating on a stir plate.

3. Once solution is clear, dispense 7ml aliquots into 20x150mm test tubes.

4. Allow gels to solidify, then cover tubes with parafilm and store at 2-5°C.
SPECIES TANK BUFFER

standard batch size: 15L

Reagents

sodium barbiturate
diethyl barbituric acid (barbital)
calcium lactate

Procedure

1. Dissolve 131.4g sodium barbiturate, 20.7g barbital, and 5.7g calcium lactate in 2-4L distilled water.

2. Transfer solution to a carboy and dilute to 15L with distilled water.

3. Adjust the pH to 8.6, if necessary, with either sodium hydroxide (to increase pH) or hydrochloric acid (to lower pH).
TAKAYAMA REAGENT

standard batch size: 100ml

Reagents

dextrose (glucose)
sodium hydroxide
pyridine

Procedure

1. Dissolve 0.5g dextrose in 5ml distilled water.
2. Dissolve 1.0g sodium hydroxide in 10ml distilled water.
3. Transfer both the dextrose and sodium hydroxide solutions to a flask and add 20ml pyridine.
4. Dilute solution to 100ml with distilled water.
5. Store in a brown glass bottle at 2-5°C.
UREA DIFFUSION TEST PLATES

standard batch size: 15 plates

Reagents

bromothymol blue solution (BTB)

agarose (Sigma type I or equivalent)

urease solution

Procedure

1. Dissolve 4.5g agarose in 450ml boiling distilled water.

2. Add 4.5ml bromothymol blue solution to the boiling agarose solution. The bromothymol blue solution is prepared by dissolving 1.5g BTB in 100ml distilled water and one drop of phosphoric acid diluted 1:10 with distilled water.

3. Allow solution to cool to 50°C.

4. Add 5ml of urease solution (300U/100ml distilled water) to the gel solution.

5. Dispense 30ml aliquots of the gel solution in 10cmF SQuArS C Our petri dishes and allow to solidify.
UREA DIFFUSION BLANK PLATES

stand batch size: 15 plates

Reagents

bromothymol blue solution (BTB)

agarose (Sigma type I or equivalent)

Procedure

1. Dissolve 4.5g agarose e is 450ml boiling distilled water.

2. Add 4.5ml bromothymol blue solution to the boiling agarose solution. The bromothymol blue solution is prepared by dissolving 1.5g BTB in 100ml distilled water and one drop of phosphoric acid diluted 1:10 with distilled water.

3. Dispense 30ml aliquots of the gel solution in 10cmF squar&COUR petri dishes and allow to solidify.
II

Forensic Molecular Biology

Solutions Manual - HLA-DQα

Version 1.0
S001 SDS, 20%  

standard batch size: 1 L

INGREDIENTS | final concentration | amount
-------------|---------------------|--------
RM007 sodium dodecyl sulfate | 20 % | 200 ± 5 g

PROCEDURE

CAUTION: AN AEROSOL MASK OR FUME HOOD MUST BE USED WHEN MAKING THIS SOLUTION. WEAR GOGGLES FOR EYE PROTECTION.

Warm approximately 750 mL distilled water on a stirring hot plate. Add a fraction of the SDS, allowing the solids to dissolve before adding more. Add the SDS until it is all in solution. When the solution is clear, bring up to volume with distilled water. Filter sterilize the warm solution. Dispense into sterile 500 mL bottles. Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>Amt</th>
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<tbody>
<tr>
<td>RM007 sodium dodecyl sulfate</td>
<td>_________</td>
<td>_______</td>
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</table>

made by: ___________________________  date: ___________
S002 SSPE, 20X

standard batch size: 4 L

INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM003 EDTA</td>
<td>20. mM</td>
<td>29.8 ± 0.7 g</td>
</tr>
<tr>
<td>RM004 sodium hydroxide, 10N (guideline)</td>
<td>----</td>
<td>40 ± 5 ml</td>
</tr>
<tr>
<td>RM005 sodium chloride</td>
<td>3.6 M</td>
<td>840 ± 10 g</td>
</tr>
<tr>
<td>RM006 sodium phosphate, monobasic</td>
<td>200 mM</td>
<td>110 ± 3 g</td>
</tr>
</tbody>
</table>

PROCEDURE

Dissolve the EDTA in approximately 3 liters distilled water.

Adjust the pH to approximately 6.0 with 10N sodium hydroxide to help dissolve the EDTA.

Add the sodium phosphate first and then the sodium chloride.

Adjust the pH to 7.4 with 10N sodium hydroxide (about 40 ml).

Adjust the final volume to 4 liters with deionized water.

Measure and record the final pH.

Dispense into 1 L bottles.

Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM003 EDTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM004 sodium hydroxide, 10N</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM005 sodium chloride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM006 sodium phosphate, monobasic</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
QUALITY CONTROL

final pH: ____________________________ specification 7.4 ± 0.2
made by: ____________________________ date: _______
**S003 DQα CITRATE BUFFER**

lot number: _____

standard batch size: 4 L

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM001 trisodium citrate</td>
<td>-----</td>
<td>73.6 ± 0.1 g</td>
</tr>
<tr>
<td>RM002 citric acid (guideline)</td>
<td>-----</td>
<td>24. ± 1. g</td>
</tr>
</tbody>
</table>

**PROCEDURE**

Dissolve the sodium citrate in approximately 3 liters distilled water.

Adjust the pH to 5.0 by addition of citric acid (approximately 24 g).

Adjust the final volume to 4 liters with distilled water.

Mix well.

Measure and record the final pH.

Dispense into a 4 L bottle.

Store at room temperature.

**DATA LOG**

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM001 trisodium citrate</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>RM002 citric acid</td>
<td>_______</td>
<td>_______</td>
</tr>
</tbody>
</table>

**QUALITY CONTROL**

final pH: __________________________ specification 5.0 ± 0.2

QC003 DQα hybridization

made by: ___________________________ date: _________

April 2, 1992  II-4
S004 DQα HYBRIDIZATION SOLUTION

lot number: ____

standard batch size: 4 L

INGREDIENTS

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S002 SSPE, 20X</td>
<td>5.0 X</td>
<td>1000 ± 10 ml</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>0.50 %</td>
<td>100 ± 1 ml</td>
</tr>
</tbody>
</table>

PROCEDURE

Combine the SSPE and 2.9 L distilled water in a 4 L flask.

Add the SDS.

Warm the solution until all solids are dissolved.

Mix well.

Dispense into 1 L bottles.

Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>amount</th>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>S002 SSPE, 20X</td>
<td>_______</td>
<td>_______</td>
<td>___</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>_______</td>
<td>_______</td>
<td>___</td>
</tr>
</tbody>
</table>

QUALITY CONTROL

QC003 DQα hybridization

made by: ____________________________ date: ______

April 2, 1992 II-5
S005 DQα WASH SOLUTION

lot number: _____

standard batch size: 4 L

INGREDIENTS

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S002 SSPE, 20X</td>
<td>2.5 X</td>
<td>500 ± 10 ml</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>0.10 %</td>
<td>20 ± 1 ml</td>
</tr>
</tbody>
</table>

PROCEDURE

Measure 20 ml 20% SDS in a 50 ml graduated cylinder.

Raise the volume of the SDS solution to 50 ml by adding 30 ml distilled water.

Pour the SDS into a 4 L bottle.

Add 500 ml SSPE and 3450 ml distilled water.

Cap and mix well by inverting.

Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th></th>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>S002 SSPE, 20X</td>
<td>_______</td>
<td>_______</td>
<td>___</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>_______</td>
<td>_______</td>
<td>___</td>
</tr>
</tbody>
</table>

QUALITY CONTROL

QC003 DQα hybridization

made by: ___________________________ date: _______

April 2, 1992  II-6
S009 EDTA, 0.5M

standard batch size: 500 ml

INGREDIENTS

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM003 EDTA</td>
<td>0.50 M</td>
<td>93 ± 1 g</td>
</tr>
<tr>
<td>RM004 sodium hydroxide, 10N</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

PROCEDURE

Add the EDTA to approximately 250 ml distilled water.
Adjust the pH to 8.0 with sodium hydroxide solution.
Mix well.
When the EDTA is dissolved, adjust the pH to 8.0.
Bring up to volume with distilled water.
Check and record the final pH.
Dispense into 125 ml bottles.
Autoclave at 250°F for 20 minutes.
Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM003 EDTA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM004 sodium hydroxide, 10N</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

QUALITY CONTROL

final pH: ________________ specification: 8.0 ± 0.1

made by: ____________________________ date: ________

April 2, 1992

II-7
S014 Proteinase-K Enzyme, 10mg/ml

standard batch size: 10 ml

lot number: __________________

Ingredients

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM119 proteinase-K,</td>
<td>10 mg/ml</td>
<td>100 ± 1 mg</td>
</tr>
<tr>
<td>lyophilized</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Procedure

Add 10 ml sterile, distilled water to one bottle (100 mg) lyophilized proteinase-K enzyme.

Mix by slowly inverting until completely reconstituted.

Dispense 500 ul aliquots into 1.5 ml eppendorf tubes.

Store at -20°C.

Data Log

<table>
<thead>
<tr>
<th>ingredient</th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM119 proteinase-K,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lyophilized</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quality Control

QA004 DQα differential extraction

made by: ____________________________ date: ____________________________

March 9, 1993 II-8
S014 PROTEINASE-K ENZYME, 10MG/ML

standard batch size: 10 ml

INGREDIENTS

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM119 proteinase-k, lyophilized</td>
<td>10 mg/ml</td>
<td>100 ± 1 mg</td>
</tr>
</tbody>
</table>

PROCEDURE

Add 10 ml sterile, distilled water to one bottle (100 mg) lyophilized proteinase-k enzyme.

Mix by slowly inverting until completely reconstituted.

Dispense 500 ul aliquots into 1.5 ml eppendorf tubes.

Store at -20°C.

DATA LOG

<table>
<thead>
<tr>
<th></th>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM119 proteinase-k, lyophilized</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ________________________________ date: ______
S018 ANALYTICAL GEL LOADING BUFFER

lot number: ______
standard batch size: 100 ml

INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM020 bromophenol blue</td>
<td>0.25%</td>
<td>0.25 ± 0.01 g</td>
</tr>
<tr>
<td>RM217 xylene cyanol</td>
<td>0.25%</td>
<td>0.25 ± 0.01 g</td>
</tr>
<tr>
<td>RM040 ficoll 400</td>
<td>12.5%</td>
<td>12.5 ± 0.1 g</td>
</tr>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>50. mM</td>
<td>10.0 ± 0.1 ml</td>
</tr>
<tr>
<td>RM083 TAE, 10X</td>
<td>5.0 X</td>
<td>50.0 ± 0.5 ml</td>
</tr>
</tbody>
</table>

PROCEDURE

Combine the TAE, EDTA, and ficoll.
Mix well. The solution may need to be heated gently to dissolve the ficoll.
Add the bromophenol blue and xylene cyanol.
Mix well.
When all the solids are dissolved, bring up to volume using distilled water.
Filter sterilize.
Dispense 1.5 ml aliquots into 1.5 ml eppendorf tubes.
Store at -20°C.

DATA LOG

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM020 bromophenol blue</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>RM217 xylene cyanol</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>RM040 ficoll 400</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

April 2, 1992 II-9
Initials: RC Date: 11/12/92
RM083 TAE, 10X

made by: ____________________________ date: ___

April 2, 1992 II-10
S022 CHELEX, 5%  
standard batch size: 500 ml

INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM027 chelex 100</td>
<td>5. %</td>
<td>25 ± 2 g</td>
</tr>
<tr>
<td>S059 sterile water (guideline)</td>
<td>---</td>
<td>450 ± 50 ml</td>
</tr>
</tbody>
</table>

PROCEDURE

Filter sterilize approximately 600 ml distilled water.

Pour the water into a 500 ml bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the chelex to a volume of 500 ml using the graduation markings on the disposable filter container.

Mix on a magnetic stirrer.

While the stock solution is mixing, aliquot 10 ml each into 15 ml centrifuge tubes.

Store at 2-8°C.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM027 chelex 100</td>
<td>_____</td>
<td>_____</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>_____</td>
<td>_____</td>
</tr>
</tbody>
</table>

QUALITY CONTROL

QC001 DQα extraction

made by: ____________________________ date: _____

April 2, 1992

II-11
S059 STERILE WATER

standard batch size: 500 ml

PROCEDURE
Filter sterilize 500 ml of distilled water.
Pour into sterile, 125 ml bottles.
Autoclave at 250°F for 30 minutes.
Store at room temperature.

made by: ____________________________ date: ____
S059 STERILE WATER

standard batch size: 500 ml

PROCEDURE

Filter sterilize 500 ml of distilled water.

Aliquot 10 ml each into 15 ml centrifuge tubes.

Autoclave at 250°F for 30 minutes.

Store at room temperature.

made by: ______________________________ date: ______
S079 HYDROGEN PEROXIDE, 3%  
lot number: ______

standard batch size: 80 X 0.5 ml

INGREDIENTS          final concentration     amount

RM284 hydrogen peroxide, 3%  3 %            0.5 ml
(guideline)

PROCEDURE

Aliquot approximately 0.5 ml of hydrogen peroxide into 1.5 ml microcentrifuge tubes.

Label each tube with "H₂O₂" and the lot number.

Store at 4°C in the dark.

DATA LOG          source     lot     amt

RM284 hydrogen peroxide, 3%  ________  ________  ____

QUALITY CONTROL

QC003 DQα hybridization

made by: ___________________________ date: _____

April 2, 1992
II-13
SODIUM ACETATE, 1M

lot number: ______

standard batch size: 100 mL

INGREDIENTS

<table>
<thead>
<tr>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM059 sodium acetate, anhydrous</td>
<td>1.0 M</td>
</tr>
<tr>
<td>RM093 acetic acid, glacial</td>
<td>------</td>
</tr>
</tbody>
</table>

PROCEDURE

Add the sodium acetate to approximately 75 ml distilled water.
Mix well.
Adjust the pH to 5.2 with glacial acetic acid.
Bring up to volume with distilled water.
Measure and record the final pH.
Dispense into a 100 ml bottle.
Autoclave at 250°F for 30 minutes.
Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM059 sodium acetate, anhydrous</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>RM093 acetic acid, glacial</td>
<td>------</td>
<td>------</td>
</tr>
</tbody>
</table>

made by: ________________________________ date: ______

April 2, 1992           II-14
SO81 DTT, 1M

standard batch size: 5 ml

INGREDIENTS

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM101 dithiothreitol</td>
<td>1.0 M</td>
<td>0.77 ± 0.04 g</td>
</tr>
<tr>
<td>S080 sodium acetate, 1M</td>
<td>10. mM</td>
<td>50 ± 3 µl</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

PROCEDURE

Add the DTT to approximately 4 ml sterile, distilled water in a 15 ml centrifuge tube.

Mix well.

When the DTT is dissolved, add the sodium acetate solution, and bring up to volume with sterile, distilled water.

Filter sterilize.

Dispense 500 µl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

DATA LOG

<table>
<thead>
<tr>
<th></th>
<th>source</th>
<th>lot</th>
<th>amt</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM101 dithiothreitol</td>
<td>______</td>
<td>______</td>
<td>_____</td>
</tr>
<tr>
<td>S080 sodium acetate, 1M</td>
<td>______</td>
<td>______</td>
<td>_____</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>______</td>
<td>______</td>
<td>_____</td>
</tr>
</tbody>
</table>

made by: ________________________________ date: ______

April 2, 1992
S007 TRIS-HCl, 1M - PH 7.6  
lot number: ____________________

standard batch size: 250 ml

**INGREDIENTS**

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM073 TRIS</td>
<td>1.00 M</td>
<td>30.3 ± 0.1 g</td>
</tr>
<tr>
<td>RM096 hydrochloric acid</td>
<td>-------</td>
<td>-------</td>
</tr>
</tbody>
</table>

**PROCEDURE**

Add the TRIS to approximately 200 ml distilled water.
Mix well.
Adjust the pH to 7.6 with concentrated hydrochloric acid.
Bring up to final volume with distilled water.
Measure and record the final pH.

Prepare a 1:100 dilution (10 mM TRIS-HCl) by mixing 1 ml TRIS-HCl solution and 99 ml distilled water.

Measure and record the pH of the dilution.
Dispense the 1M TRIS-HCl into 125 ml bottles.
Autoclave at 250°F for 20 minutes.
Store at room temperature.

**DATA LOG**

<table>
<thead>
<tr>
<th></th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM073 TRIS</td>
<td>________</td>
<td>________</td>
<td>________</td>
</tr>
<tr>
<td>RM096 hydrochloric acid</td>
<td>________</td>
<td>________</td>
<td>________</td>
</tr>
</tbody>
</table>

**final pH:** ____________________  specification: 7.6 ± 0.1

**1:100 pH:** ____________________  specification: 7.6 ± 0.1

April 14, 1992  II-16
MAGNESIUM CHLORIDE, 1M

standard batch size: 250 ml

INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM046 magnesiuim chloride, hexahydrate</td>
<td>1.00 M</td>
<td>50.8 ± 0.3 g</td>
</tr>
</tbody>
</table>

PROCEDURE

Dissolve the magnesium chloride in approximately 200 ml distilled water.

Mix well.

When the magnesium chloride has dissolved, bring up to the final volume with distilled water.

Dispense into 125 ml bottles.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>Source</th>
<th>Lot</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM046</td>
<td></td>
<td></td>
</tr>
<tr>
<td>magnesiuim chloride, hexahydrate</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ___________________________ date: ________________
CELL LYSIS BUFFER (CLB)

lot number: __________________

standard batch size: 2 L

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM068 sucrose</td>
<td>320 mM</td>
<td>219 ± 3 g</td>
</tr>
<tr>
<td>S007 TRIS-HCl, 1M - pH 7.6</td>
<td>10. mM</td>
<td>20 ± 1 ml</td>
</tr>
<tr>
<td>S008 magnesium chloride, 1M</td>
<td>5. mM</td>
<td>10 ± 1 ml</td>
</tr>
<tr>
<td>RM075 triton X-100</td>
<td>1.0 %</td>
<td>20 ± 1 ml</td>
</tr>
</tbody>
</table>

PROCEDURE

Dissolve the sucrose in approximately 1.5 L distilled water.

Add the TRIS, magnesium chloride, and triton to the solution.

Mix well.

Just the volume to 2 L with distilled water.

Filter sterilize.

Dispense into sterile 500 ml bottles.

Store at 2–8°C.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM068 sucrose</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S007 TRIS-HCl, 1M - pH 7.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S008 magnesium chloride, 1M</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM075 triton X-100</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ____________________________ date: ____________________________

April 14, 1992 II-19
S011 PROTEIN LYSIS BUFFER (PLB)  
lot number: ________________
standard batch size: 2 L

INGREDIENTS        final concentration amount

S036 TRIS-HCl, 1M - pH 7.4  10 mM  20 ± 1 ml
S009 EDTA, 0.5M  10 mM  40 ± 2 ml
S012 sodium chloride, 5M  10 mM  4.0 ± 0.2 ml

PROCEDURE

Add the TRIS, EDTA, and sodium chloride to approximately 1.5 L distilled water. Raise to the final volume with distilled water.

Mix well.

Dispense into 500 ml bottles.

Autoclave at 250°F for 30 minutes.

Store at 2-8°C.

DATA LOG

source    lot    amount

S036 TRIS-HCl, 1M - pH 7.4
S009 EDTA, 0.5M
S012 sodium chloride, 5M

made by: ___________________________ date: ___________________________
SODIUM CHLORIDE, 5M

standard batch size: 6 L

lot number: ______________

INGREDIENTS

<table>
<thead>
<tr>
<th></th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM005 sodium chloride</td>
<td>5.0 M</td>
<td>1750 ± 10 g</td>
</tr>
</tbody>
</table>

PROCEDURE

Slowly add the sodium chloride to approximately 2 L distilled water.

Raise the volume to just under 6 L so that the sodium chloride will go into solution.

Mix well.

Bring up to volume with distilled water.

Large volumes used for denaturation and neutralization solutions need not be autoclaved. Sodium chloride solution for other uses must be dispensed into 500 ml bottles and autoclaved at 250°F for 30 minutes.

Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th></th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM005 sodium chloride</td>
<td>_________</td>
<td>_________</td>
<td>_________</td>
</tr>
</tbody>
</table>

made by: _________________________ date: ___________________
**S020 YIELD CALIBRATORS**

lot number: ____________________

standard batch size: 5 X 400μl each

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S039 TE, 1X</td>
<td>1 X</td>
<td>_______</td>
</tr>
<tr>
<td>RM148 lambda DNA</td>
<td>_______</td>
<td>140 ± 10 μg</td>
</tr>
<tr>
<td>S021 yield gel loading buffer</td>
<td>1.25 X</td>
<td>3.0 ± 0.5 ml</td>
</tr>
</tbody>
</table>

**CALCULATIONS**

stock solution

<table>
<thead>
<tr>
<th>final DNA concentration</th>
<th>final volume</th>
<th>initial DNA concentration</th>
<th>volume lambda DNA</th>
<th>volume 1X TE</th>
</tr>
</thead>
<tbody>
<tr>
<td>50ng/μl</td>
<td>2800μl</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
</tbody>
</table>

**Calibrators**

<table>
<thead>
<tr>
<th>final DNA concentration</th>
<th>final volume</th>
<th>stock DNA concentration</th>
<th>volume stock DNA</th>
<th>volume water</th>
<th>volume buffer</th>
</tr>
</thead>
<tbody>
<tr>
<td>A 300ng/10μl</td>
<td>2000μl</td>
<td>50ng/μl</td>
<td>1200μl</td>
<td>300μl</td>
<td>500μl</td>
</tr>
<tr>
<td>B 200ng/10μl</td>
<td>2000μl</td>
<td>50ng/μl</td>
<td>800μl</td>
<td>700μl</td>
<td>500μl</td>
</tr>
<tr>
<td>C 100ng/10μl</td>
<td>2000μl</td>
<td>50ng/μl</td>
<td>400μl</td>
<td>1100μl</td>
<td>500μl</td>
</tr>
<tr>
<td>D 50ng/10μl</td>
<td>2000μl</td>
<td>50ng/μl</td>
<td>200μl</td>
<td>1300μl</td>
<td>500μl</td>
</tr>
<tr>
<td>E 25ng/10μl</td>
<td>2000μl</td>
<td>50ng/μl</td>
<td>100μl</td>
<td>1400μl</td>
<td>500μl</td>
</tr>
<tr>
<td>F 10ng/10μl</td>
<td>2000μl</td>
<td>50ng/μl</td>
<td>40μl</td>
<td>1460μl</td>
<td>500μl</td>
</tr>
</tbody>
</table>

**PROCEDURE**

Each lot of yield calibrators is prepared as a batch of five sets. Each batch requires 2800 μl of 50 ng/μl stock lambda DNA solution.

Record the concentration in ng/μl of the lambda DNA received from the manufacturer under initial DNA concentration.

April 14, 1992

II-22
S020 YIELD CALIBRATORS

lot number: ____________________________

page 2 of 2

Calculate the volume of lambda DNA required for the stock solution according to equation 1.

\[
\text{volume lambda DNA) = (final DNA concentration)(final volume) - (initial DNA concentration)}
\] equation 1

PROCEDURE

Calculate the volume of 1X TE to add to the stock solution according to equation 2.

\[
\text{(volume 1X TE) = (final volume) - (volume lambda DNA)}
\] equation 2

Prepare the stock solution by diluting the lambda DNA in a sterile centrifuge tube with 1X TE.

Mix well.

Label six sterile eppendorf tubes, one for each of the six yield calibrator levels.

Pipet the appropriate amounts of DNA stock solution and sterile water into the labeled tubes. The combined volume of DNA and water is 1500 µl for each level.

Mix well.

Divide each level into five 300 µl aliquots, and dispense into labeled, sterile eppendorf tubes.

Add 100 µl of yield gel loading buffer to each tube. The final volume of each aliquot is 400 µl.

Store at -20°C.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S039 1X TE</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM148 lambda DNA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S021 yield gel loading buffer</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

April 14, 1992 II-23
S021 YIELD GEL LOADING BUFFER

lot number: ____________

standard batch size: 100 ml

INGREDIENTS

<table>
<thead>
<tr>
<th>ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM020 bromophenol blue</td>
<td>0.25%</td>
<td>0.25 ± 0.01 g</td>
</tr>
<tr>
<td>RM217 xylene cyanol</td>
<td>0.25%</td>
<td>0.25 ± 0.01 g</td>
</tr>
<tr>
<td>RM040 ficoll 400</td>
<td>12.5%</td>
<td>12.5 ± 0.1 g</td>
</tr>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>50. mM</td>
<td>10.0 ± 0.1 ml</td>
</tr>
<tr>
<td>RM083 TAE, 10X</td>
<td>5.0 X</td>
<td>50.0 ± 0.5 ml</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>0.20 %</td>
<td>1.00 ± 0.02 mL</td>
</tr>
</tbody>
</table>

PROCEDURE

Combine the TAE, EDTA, SDS, and ficoll.

Mix well. The solution may need to be heated gently to dissolve the ficoll.

Add the bromophenol blue and xylene cyanol.

Mix well.

When all the solids are dissolved, bring up to volume using distilled water.

Filter sterilize.

Dispense 1.5 ml aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

DATA LOG

<table>
<thead>
<tr>
<th>ingredient</th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM020 bromophenol blue</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>RM217 xylene cyanol</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>RM040 ficoll 400</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>RM083 TAE, 10X</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
</tbody>
</table>

April 14, 1992  II-25
S021 YIELD GEL LOADING BUFFER

standard batch size: 100 ml

lot number: ________________

INGREDIENTS

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM020 bromophenol blue</td>
<td>0.25%</td>
<td>0.25 ± 0.01 g</td>
</tr>
<tr>
<td>RM217 xylene cyanol</td>
<td>0.25%</td>
<td>0.25 ± 0.01 g</td>
</tr>
<tr>
<td>RM040 ficoll 400</td>
<td>12.5%</td>
<td>12.5 ± 0.1 g</td>
</tr>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>50. mM</td>
<td>10.0 ± 0.1 ml</td>
</tr>
<tr>
<td>RM083 TAE, 10X</td>
<td>5.0 X</td>
<td>50.0 ± 0.5 ml</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>0.20 %</td>
<td>1.00 ± 0.02 mL</td>
</tr>
</tbody>
</table>

PROCEDURE

Combine the TAE, EDTA, SDS, and ficoll.

Mix well. The solution may need to be heated gently to dissolve the ficoll.

Add the bromophenol blue and xylene cyanol.

Mix well.

When all the solids are dissolved, bring up to volume using distilled water.

Filter sterilize.

Dispense 1.5 ml aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

DATA LOG

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM020 bromophenol blue</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>RM217 xylene cyanol</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>RM040 ficoll 400</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>RM083 TAE, 10X</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
<tr>
<td>S001 20% SDS</td>
<td>______</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

April 14, 1992				II-27
LAMBDA HIND III, 20NG/μL

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>initial concentration (ng/μl)</th>
<th>initial volume (μl)</th>
<th>final concentration</th>
<th>final volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM155 lambda Hind III fragments</td>
<td></td>
<td></td>
<td>20 ng/μl</td>
<td></td>
</tr>
<tr>
<td>S021 yield gel loading buffer</td>
<td>5 X</td>
<td></td>
<td>1 X</td>
<td>----</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>----</td>
<td></td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

CALCULATIONS

Record the initial concentration in ng/μl and the initial volume in μl of the lambda Hind III DNA received from the manufacturer.

Calculate the final volume according to equation 1.

\[
(final \ volume) = \frac{(initial \ DNA \ concentration)(initial \ DNA \ volume)}{20 \ ng/μl}
\]

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

\[
(buffer \ volume) = 0.2(final \ volume)
\]

Calculate the amount of sterile water to be added according to equation 3.

\[
(water \ volume) = 0.8(final \ volume) - (initial \ DNA \ volume)
\]

Record the buffer and water volumes above.

April 14, 1992

II-29
To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water. The sum of the initial volumes must be equal to the calculated final volume.

**PROCEDURE**

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500 µl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

**DATA LOG**

<table>
<thead>
<tr>
<th>Source</th>
<th>Lot</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>λ155 lambda Hind III fragments</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S021 yield gel loading buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S059 sterile water</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ___________________________ date: ________________

April 14, 1992 II-30
S036 TRIS-HCl, 1M – PH 7.4

lot number: ________________

standard batch size: 250 ml


ingredients

<table>
<thead>
<tr>
<th>ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM073 TRIS</td>
<td>1.00 M</td>
<td>30.3 ± 0.1 g</td>
</tr>
<tr>
<td>RM096 hydrochloric acid</td>
<td>-------</td>
<td>----</td>
</tr>
</tbody>
</table>

PROCEDURE

Add the TRIS to approximately 200 ml distilled water.
Mix well.
Adjust the pH to 7.4 with concentrated hydrochloric acid.
Bring up to final volume with distilled water.
Measure and record the final pH.
Prepare a 1:100 dilution (10 mM TRIS-HCl) by mixing 1 ml TRIS-HCl solution and 99 ml distilled water.
Measure and record the pH of the dilution.
Dispense the 1M TRIS-HCl into 125 ml bottles.
Autoclave at 250°F for 20 minutes.
Store at room temperature.

DATA LOG

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM073 TRIS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM096 hydrochloric acid</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

final pH: __________________________ specification: 7.4 ± 0.1
1:100 pH: __________________________ specification: 7.4 ± 0.1

April 14, 1992
II-31
S042 PHI-X Marker

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>initial concentration (ng/μl)</th>
<th>initial volume (μl)</th>
<th>final concentration</th>
<th>final volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM156 phi-X-174, Hae III fragments</td>
<td></td>
<td></td>
<td>50 ng/μl</td>
<td></td>
</tr>
<tr>
<td>S018 analytical gel loading buffer</td>
<td>5 X</td>
<td></td>
<td>1 X</td>
<td>----</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>-----</td>
<td></td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

CALCULATIONS

Record the initial concentration in ng/μl and the initial volume in μl of the phi-X-174 Hae III received from the manufacturer.

Calculate the final volume according to equation 1.

\[(\text{final volume}) = \frac{\text{(initial DNA concentration)} \times \text{(initial DNA volume)}}{50 \text{ ng/μl}}\] equation 1

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

\[(\text{buffer volume}) = 0.2(\text{final volume})\] equation 2

Calculate the amount of sterile water to be added according to equation 3.

\[(\text{water volume}) = 0.8(\text{final volume}) - (\text{initial DNA volume})\] equation 3

Record the buffer and water volumes above.

April 14, 1992

II-33
To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

**PROCEDURE**

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 500 μl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

<table>
<thead>
<tr>
<th>DATA LOG</th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>phi-X-174 Hae III fragments</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S018 analytical gel loading buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S059 sterile water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ___________________________  date: ____________________

April 14, 1992  II-34
S050 CALIBRATION CONTROL

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>initial concentration (ng/μl)</th>
<th>initial volume (μl)</th>
<th>final concentration</th>
<th>final volume (μl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM221 K562 DNA</td>
<td></td>
<td></td>
<td>5 ng/μl</td>
<td></td>
</tr>
<tr>
<td>S021 yield gel loading buffer</td>
<td>5 X</td>
<td></td>
<td>1 X</td>
<td>----</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>-----</td>
<td></td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

CALCULATIONS

Record the initial concentration in ng/μl and the initial volume in μl of the K562 DNA received from the manufacturer.

Calculate the final volume according to equation 1.

\[
\text{final volume} = \frac{(\text{initial DNA concentration})(\text{initial DNA volume})}{(5 \text{ ng/μl})}
\] equation 1

Record the final volume above. The final volume is the total batch size.

Calculate the amount of buffer to be added according to equation 2.

\[
\text{buffer volume} = 0.2(\text{final volume})
\] equation 2

Calculate the amount of sterile water to be added according to equation 3.

\[
\text{water volume} = 0.8(\text{final volume}) - (\text{initial DNA volume})
\] equation 3

Record the buffer and water volumes above.

April 14, 1992
To check the calculations, add together the initial volumes of DNA, loading buffer, and sterile water.

The sum of the initial volumes must be equal to the calculated final volume.

**PROCEDURE**

Combine the DNA, loading buffer, and sterile water.

Mix well.

Using sterile pipet tips, dispense 200 μl aliquots into sterile 1.5 ml eppendorf tubes.

Store at -20°C.

<table>
<thead>
<tr>
<th>DATA LOG</th>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM221 K562 DNA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3021 yield gel loading</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>buffer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S059 sterile water</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ________________________________ date: ____________________

April 14, 1992

II-36
S064 CELL PELLET CONTROL

<table>
<thead>
<tr>
<th>INGREDIENTS</th>
<th>concentration of cells</th>
<th>total volume (ml)</th>
<th>cells per aliquot</th>
<th>aliquot volume (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM243 K562 cells</td>
<td></td>
<td></td>
<td>1.10^6</td>
<td></td>
</tr>
<tr>
<td>S034 phosphate buffered saline (PBS)</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
</tbody>
</table>

CALCULATIONS

Record the concentration of K562 cells in the suspension received from the manufacturer.

Record the total volume. This is the batch size.

Calculate the volume (in ml) which yields 1.10^6 cells according to equation 1.

\[
\text{(aliquot volume)} = \frac{1.10^6 \text{cells}}{\text{concentration of cells}} \quad \text{equation 1}
\]

The aliquot volume must fit into a 1.5 ml eppendorf tube. The concentration of the cell suspension may have to be adjusted.

If the cell concentration is too low, the cells may be spun at 180 g for 5 minutes at 4°C. Remove the excess media to give the desired concentration.

If the cell concentration is too high, PBS may be added to reach the desired concentration. After adding PBS, make sure the cells are well suspended before aliquoting.

Record the calculated aliquot volume.

PROCEDURE

The following steps must be done on ice or at 4°C.

Bring the cell suspension up to the desired final volume.

Suspend the cells evenly by pipetting up and down or by gently inverting the container.

Add aliquots of cell suspension to 1.5 ml eppendorf tubes.

in the tubes at 180 g for 1 minute at 4°C, and remove the excess supernatant.

April 14, 1992

II-37
The tubes can be aliquoted and spun in sets of 52. Each set should be packaged separately in a seal-a-meal bag, labeled with the lot number and numbered sequentially.

Store the bags at -70°C.

DATA LOG  

<table>
<thead>
<tr>
<th>Source</th>
<th>Lot</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM243 K562 cells</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S034 phosphate buffered saline</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

made by: ____________________  date: ____________________
S034 Phosphate Buffered Saline (PBS)  
lot number: ____________________

standard batch size: 4 L

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM005 sodium chloride</td>
<td>137 mM</td>
<td>32.0 ± 0.1 g</td>
</tr>
<tr>
<td>RM053 potassium chloride</td>
<td>3.0 mM</td>
<td>0.90 ± 0.01 g</td>
</tr>
<tr>
<td>RM065 sodium phosphate, dibasic</td>
<td>6.0 mM</td>
<td>3.41 ± 0.03 g</td>
</tr>
<tr>
<td>RM056 potassium phosphate, monobasic</td>
<td>1.5 mM</td>
<td>0.82 ± 0.02 g</td>
</tr>
</tbody>
</table>

Procedure

Add all the components to approximately 3 L distilled water.

Mix well.

Adjust the pH to 7.5.

Bring up to the final volume with distilled water.

Measure and record the final pH.

Dispense into 50 ml centrifuge tubes.

Autoclave at 250°F for 20 minutes.

Store at room temperature.

Data Log

<table>
<thead>
<tr>
<th>source</th>
<th>lot</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM005 sodium chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM053 potassium chloride</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM065 sodium phosphate, dibasic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RM056 potassium phosphate, monobasic</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quality Control

final pH: ___________________________  spec: 7.5 ± 0.1

004 DQα differential extraction

made by: ___________________________  date: ______________________

March 9, 1993  II-40
S082 Chelex, 20%  
lot number: ______________

Standard batch size: 500 ml

**Ingredients**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM027 chelex 100</td>
<td>20. %</td>
<td>100 ± 2 g</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>---</td>
<td>450 ± 50 ml (guideline)</td>
</tr>
</tbody>
</table>

**Procedure**

Filter sterilize approximately 600 ml distilled water.

Pour the water into a 500 ml bottle.

Save the bottom container from the disposable filter unit.

Autoclave the water at 250°F for 30 minutes.

Add the chelex to the bottom container of the filter unit.

Allow the water to cool after autoclaving.

Add sterile water to the chelex to a volume of 500 ml using the graduation markings on the disposable filter container.

Mix on a magnetic stir plate.

While the stock solution is mixing, aliquot 10 ml each into 15 ml centrifuge tubes.

Store at 2-8°C.

**Data Log**

<table>
<thead>
<tr>
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</tr>
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<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
</tbody>
</table>

**Quality Control**

QC004 DQα differential extraction

made by: ____________________________  date: ____________________________

March 9, 1993  II-41
Initials: [Signature]  Date: 3/0/93

S093 DTT, 1M  lot number: _____________

Standard batch size: 20 ml

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>RM101 dithiothreitol</td>
<td>1.0 M</td>
<td>3.1 ± 0.2 g</td>
</tr>
<tr>
<td>S059 sterile water</td>
<td>______</td>
<td>______</td>
</tr>
</tbody>
</table>

Procedure

Add the DTT to approximately 15 ml sterile, distilled water in a 50 ml centrifuge tube.

Mix well.

When the DTT is dissolved, bring up to volume with sterile, distilled water.

Filter sterilize.

Dispense 250 µl aliquots into sterile 0.5 ml eppendorf tubes.

Store at -20°C.

Data Log

<table>
<thead>
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</table>

Quality Control

QA004 DQα differential extraction

made by: ________________________________  date: __________________

March 9, 1993  II-42
S094 Digest Buffer  
Standard batch size:  6 L  

lot number:  

Ingredients 

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>final concentration</th>
<th>amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>S009 EDTA, 0.5M</td>
<td>10. mM</td>
<td>120 ± 6 ml</td>
</tr>
<tr>
<td>S036 TRIS-HCl, 1M-pH 7.4</td>
<td>10. mM</td>
<td>60 ± 3 ml</td>
</tr>
<tr>
<td>S012 sodium chloride, 5M</td>
<td>50. mM</td>
<td>60 ± 1 ml</td>
</tr>
<tr>
<td>S001 SDS, 20%</td>
<td>2.0 %</td>
<td>600 ± 15 ml</td>
</tr>
<tr>
<td>RM096 hydrochloric acid</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Procedure

Add the EDTA, TRIS, sodium chloride, and SDS to approximately 4 L distilled water.

Adjust the pH to 7.5.

Bring up to the final volume with distilled water.

Well.

Measure and record the final pH.

Aliquot into 50 ml centrifuge tubes.

Store at room temperature.

Data Log 

<table>
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</tr>
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<td>_______</td>
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<td>RM096 hydrochloric acid</td>
<td>_______</td>
<td>_______</td>
<td>_______</td>
</tr>
</tbody>
</table>

Quality Control

final pH:  

specification:  7.5 ± 0.1

04 differential extraction

made by:  
date:  

March 9, 1993  

II-43