Department of Forensic Biology

Solutions Manual

Version 1.0

April 2, 1992

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Forensic Biochemistry & Hematology Laboratory

Solutions Manual

Version 1.0

April 2, 1992

Initials: RC Date: 4/2/92

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.

Initials: ES Date: 4/2/92

ACID PHOSPHATASE REACTION BUFFER

standard batch size: 200ml

Reagents

citric acid

sodium hydroxide

Procedure

1. Dissolve 1.92g citric acid and 0.80g sodium hyroxide in 2L distilled water.
2. Adjust the pH to 5.0, if necessary, by adding additional sodium hydroxide.
3. Store at 2-5°C.

Initials: RCJ Date: 4/2/92

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).

Initials: RG Date: 4/23/93

ALKALINE SUBSTRATE BUFFER

Standard batch size: 1 L

Reagents

diethanol amine

sodium azide

$MgCl_2$

HCl

Procedure

1. Dissolve 97 mL diethanolamine, 0.2 g sodium azide, and 0.1 g $Mgcl_2$ 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.

Initials: RCS Date: 4/2/92

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.

Initials: RCJ Date: 4/1/92

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.

Initials: RG Date: 4/2/92

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.

Initials: RCS Date: 4/23/93

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: RCJ 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.

Initials: RS Date: 11/12/93

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.

Initials: RCJ Date: 11/12/93

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.

Initials: RCJ Date: 4/2/92

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.

Initials: RS Date: 4/2/92

DITHIOTHREITOL (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: 4/2/92

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.

Initials: RCJ Date: 4/2/92

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.

Initials: LCJ Date: 4/2/92

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.

Initials: RCJ Date: 4/2/92

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.

Initials: RCJ Date: 4/4/92

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.

Initials: RCJ Date: 4/2/92

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.

Initials: RG Date: 4/2/92

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.

Initials: RCJ Date: 4/1/92

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

Reagents	Number of Plates Required				Units
	5-6	10-12	15-18	20-24	
Distilled water	20	40	60	80	ml
Sucrose	2.5	5.0	7.5	10.0	g
3% Acrylamide Premix	1.0	2.0	3.0	4.0	g
OR					
5% Acrylamide Premix	0.6	1.2	1.8	2.4	g
Acrylamide	0.4	0.8	1.2	1.6	g
OR					
Acrylamide	0.97	1.94	2.91	3.88	g
Bisacrylamide	0.03	0.06	0.09	0.12	g
Riboflavin ($1.0mg/10ml H_2O$)	150	300	450	600	ul
OR					
Ammonium Persulfate ($0.23g/5ml H_2O$)	150	300	450	600	ul

Initials: RCJ Date: 4/6/92

Table 2

System	Ampholyte(s)	Number of Plates Required				Units
		5-6	10-12	15-18	20-24	
ACP	pH 4-8	1.0	2.0	3.0	4.0	ml
	OR					
	pH 4-6	0.5	1.0	1.5	2.0	ml
	pH 6-8	0.5	1.0	1.5	2.0	ml
ESD	pH 4-6.5	1.0	2.0	3.0	4.0	ml
PGM	pH 5-7	1.0	2.0	3.0	4.0	ml
	EPPS/HEPPS	0.25	0.50	0.75	1.00	g
Hb	pH 6-8	0.5	1.0	1.5	2.0	ml
	pH 7-9	0.5	1.0	1.5	2.0	ml
	pH 3-10	0.2	0.4	1.5	2.0	ml

Initials: RCJ Date: 7/6/92

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.

Initials: RCF Date: 11/12/93

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.

Initials: RCJ Date: 10/12/94

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.

Initials: RCJ Date: 4/2/92

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.

Initials: RG Date: 4/2/92

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.

Initials: RG Date: 4/2/92

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.

Initials: RD Date: 4/2/92

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.

Initials: RCJ Date: 4/2/92

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: RCS Date: 11/12/93

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.

Initials: LCJ Date: 4/2/92

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.

Initials: RH Date: 4/23/93

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.

Initials: RG Date: 4/23/93

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.

Initials: [illegible] Date: 4/23/93

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.

Initials: RG Date: 4/2/92

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.

Initials: RS Date: 4/2/92

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.

Initials: RCS Date: 4/2/92

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).

Initials: RCJ Date: 4/6/92

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.

Initials: RCJ Date: 4/23/93

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 square COUR petri dishes and allow to solidify.

Initials: RD Date: 4/23/93

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 bromthymol 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 squareSCOUR petri dishes and allow to solidify.

II

Forensic Molecular Biology

Solutions Manual - HLA-DQα

Version 1.0

Initials: RC Date: 4/2/92

S001 SDS, 20% lot number: _____

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	**source**	**lot**	**Amt**
RM007 sodium dodecyl sulfate	_____	_____	_____

made by: _____ date: _____

Initials: RG Date: 4/2/92

S002 SSPE, 20X lot number: _____

standard batch size: 4 L

INGREDIENTS	final concentration	amount
RM003 EDTA	20. mM	29.8 ± 0.7 g
RM004 sodium hydroxide, 10N (guideline)	-----	40 ± 5 ml
RM005 sodium chloride	3.6 M	840 ± 10 g
RM006 sodium phosphate, monobasic	200 mM	110 ± 3 g

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	**source**	**lot**	**amt**
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____
RM005 sodium chloride	_____	_____	_____
RM006 sodium phosphate, monobasic	_____	_____	_____

Initials: RCJ Date: 9/14/92

QUALITY CONTROL

final pH: _____ specification 7.4 ± 0.2

made by: _____ date: _____

Initials: RG Date: 4/2/92

S003 DQα CITRATE BUFFER lot number: _____

standard batch size: 4 L

INGREDIENTS	final concentration	amount
RM001 trisodium citrate	-----	73.6 ± 0.1 g
RM002 citric acid (guideline)	-----	24. ± 1. g

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	**source**	**lot**	**amt**
RM001 trisodium citrate	_____	_____	_____
RM002 citric acid	_____	_____	_____

QUALITY CONTROL

final pH: _____ specification 5.0 ± 0.2

QC003 DQα hybridization

made by: _____ date: _____

Initials: RCJ Date: 4/2/92

S004 DQα HYBRIDIZATION SOLUTION lot number: _____

standard batch size: 4 L

INGREDIENTS	final concentration	amount
S002 SSPE, 20X	5.0 X	1000 ± 10 ml
S001 SDS, 20%	0.50 %	100 ± 1 ml

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 amount	**source**	**lot**	**amt**
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

QUALITY CONTROL

QC003 DQα hybridization

made by: _____ date: _____

Initials: RCJ Date: 4/2/92

S005 DQα WASH SOLUTION lot number: _____

standard batch size: 4 L

INGREDIENTS	final concentration	amount
S002 SSPE, 20X	2.5 X	500 ± 10 ml
S001 SDS, 20%	0.10 %	20 ± 1 ml

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	**source**	**lot**	**amt**
S002 SSPE, 20X	_____	_____	_____
S001 SDS, 20%	_____	_____	_____

QUALITY CONTROL

QC003 DQα hybridization

made by: _____ date: _____

Initials: RCJ Date: 4/2/92

S009 EDTA, 0.5M lot number:_____

standard batch size: 500 ml

INGREDIENTS	final concentration	amount
RM003 EDTA	0.50 M	93 ± 1 g
RM004 sodium hydroxide, 10N	------	------

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	**source**	**lot**	**amt**
RM003 EDTA	_____	_____	_____
RM004 sodium hydroxide, 10N	_____	_____	_____

QUALITY CONTROL

final pH: _____ specification: 8.0 ± 0.1

made by: _____ date: _____

Initials: RG Date: 3/10/93

S014 Proteinase-K Enzyme, 10mg/ml lot number: _____

standard batch size: 10 ml

Ingredients	final concentration	amount
RM119 proteinase-K, lyophilized	10 mg/ml	100 ± 1 mg

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	source	lot	amount
RM119 proteinase-K, lyophilized	_____	_____	_____

Quality Control

QA004 DQα differential extraction

made by: _____ date: _____

Initials: RCS Date: 4/2/92

S014 PROTEINASE-K ENZYME, 10MG/ML lot number: _____

standard batch size: 10 ml

INGREDIENTS	final concentration	amount
RM119 proteinase-k, lyophilized	10 mg/ml	100 ± 1 mg

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	**source**	**lot**	**amt**
RM119 proteinase-k, lyophilized	_____	_____	_____

made by: _____ date: _____

Initials: RC Date: 4/2/92

S018 ANALYTICAL GEL LOADING BUFFER lot number: _____

standard batch size: 100 ml

INGREDIENTS	final concentration	amount
RM020 bromophenol blue	0.25%	0.25 ± 0.01 g
RM217 xylene cyanol	0.25%	0.25 ± 0.01 g
RM040 ficoll 400	12.5%	12.5 ± 0.1 g
S009 EDTA, 0.5M	50. mM	10.0 ± 0.1 ml
RM083 TAE, 10X	5.0 X	50.0 ± 0.5 ml

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	**source**	**lot**	**amt**
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____

Initials: RCJ Date: 4/2/92

RM083 TAE, 10X _____ _____ _____

made by: _____ date: _____

Initials: RCJ Date: 4/2/92

S022 CHELEX, 5% lot number: _____

standard batch size: 500 ml

INGREDIENTS	final concentration	amount
RM027 chelex 100	5. %	25 ± 2 g
S059 sterile water (guideline)	---	450 ± 50 ml

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	**source**	**lot**	**amt**
RM027 chelex 100	_____	_____	_____
S059 sterile water	_____	_____	_____

QUALITY CONTROL

QC001 DQα extraction

made by: _____ date: _____

Initials: RCJ Date: 4/4/92

S059 STERILE WATER lot number: _____

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: _____

Initials: RD Date: 7/17/92

S059 STERILE WATER lot number: _____

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: _____

Initials: RCJ Date: 4/2/92

S079 HYDROGEN PEROXIDE, 3% lot number: _____

standard batch size: 80 X 0.5 ml

INGREDIENTS	final concentration	amount
RM284 hydrogen peroxide, 3% (guideline)	3 %	0.5 ml

PROCEDURE

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

Label each tube with "H_2O_2" 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: _____

Initials: RCS Date: 9/2/92

S080 SODIUM ACETATE, 1M lot number: _____

standard batch size: 100 mL

INGREDIENTS	final concentration	amount
RM059 sodium acetate, anhydrous	1.0 M	8.2 ± 0.4 g
RM093 acetic acid, glacial	-----	-----

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	**source**	**lot**	**amt**
RM059 sodium acetate, anhydrous	_____	_____	_____
RM093 acetic acid, glacial	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/2/92

S081 DTT, 1M lot number: ____

standard batch size: 5 ml

INGREDIENTS	final concentration	amount
RM101 dithiothreitol	1.0 M	0.77 ± 0.04 g
S080 sodium acetate, 1M	10. mM	50 ± 3 µl
S059 sterile water	-----	-----

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	**source**	**lot**	**amt**
RM101 dithiothreitol	_____	_____	_____
S080 sodium acetate, 1M	_____	_____	_____
S059 sterile water		_____	_____

made by: _____ date: _____

Initials: PCJ Date: 4/14/92

S007 TRIS-HCl, 1M - PH 7.6 lot number: _____

standard batch size: 250 ml

INGREDIENTS	final concentration	amount
RM073 TRIS	1.00 M	30.3 ± 0.1 g
RM096 hydrochloric acid	------	------

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	source	lot	amount
RM073 TRIS	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

final pH: _____ specification: 7.6 ± 0.1

1:100 pH: _____ specification: 7.6 ± 0.1

Initials: RCJ Date: 4/14/92

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S008 **MAGNESIUM CHLORIDE, 1M** lot number: _____

standard batch size: 250 ml

INGREDIENTS	final concentration	amount
RM046 magnesium chloride, hexahydrate	1.00 M	50.8 ± 0.3 g

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	**source**	**lot**	**amount**
RM046 magnesium chloride, hexahydrate	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S010 **CELL LYSIS BUFFER (CLB)** lot number: _____

standard batch size: 2 L

INGREDIENTS	final concentration	amount
RM068 sucrose	320 mM	219 ± 3 g
S007 TRIS-HCl, 1M - pH 7.6	10. mM	20 ± 1 ml
S008 magnesium chloride, 1M	5. mM	10 ± 1 ml
RM075 triton X-100	1.0 %	20 ± 1 ml

PROCEDURE

Dissolve the sucrose in approximately 1.5 L distilled water.

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

Mix well.

Adjust the volume to 2 L with distilled water.

Filter sterilize.

Dispense into sterile 500 ml bottles.

Store at 2-8°C.

DATA LOG	**source**	**lot**	**amount**
RM068 sucrose	_____	_____	_____
S007 TRIS-HCl, 1M - pH 7.6	_____	_____	_____
S008 magnesium chloride, 1M	_____	_____	_____
RM075 triton X-100	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

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: _____

Initials: RCJ Date: 4/14/92

5012 SODIUM CHLORIDE, 5M lot number: _____

standard batch size: 6 L

INGREDIENTS	final concentration	amount
RM005 sodium chloride	5.0 M	1750 ± 10 g

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	**source**	**lot**	**amount**
RM005 sodium chloride	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S020 YIELD CALIBRATORS lot number: _____

standard batch size: 5 X 400ul each

INGREDIENTS	final concentration	amount
S039 TE, 1X	1 X	------
RM148 lambda DNA	-----	140 ± 10 µg (guideline)
S021 yield gel loading buffer	1.25 X	3.0 ± 0.5 ml (guideline)

CALCULATIONS

stock solution

final DNA concentration	final volume	initial DNA concentration	volume lambda DNA	volume 1X TE
50ng/µl	2800µl	_____	_____	_____

Calibrators

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

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.

Initials: RCI Date: 4/14/92

S020 YIELD CALIBRATORS lot number: _____

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

$$(\text{volume lambda DNA}) = \frac{(\text{final DNA concentration})(\text{final volume})}{(\text{initial DNA concentration})} \qquad \text{equation 1}$$

PROCEDURE

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

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

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	**source**	**lot**	**amount**
S039 TE, 1X	_____	_____	_____
RM148 lambda DNA	_____	_____	_____
S021 yield gel loading buffer	_____	_____	_____

Initials: RCJ Date: 4/14/92

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S021 YIELD GEL LOADING BUFFER lot number: _____

standard batch size: 100 ml

INGREDIENTS	final concentration	amount
RM020 bromophenol blue	0.25%	0.25 ± 0.01 g
RM217 xylene cyanol	0.25%	0.25 ± 0.01 g
RM040 ficoll 400	12.5%	12.5 ± 0.1 g
S009 EDTA, 0.5M	50. mM	10.0 ± 0.1 ml
RM083 TAE, 10X	5.0 X	50.0 ± 0.5 ml
S001 SDS, 20%	0.20 %	1.00 ± 0.02 mL

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	**source**	**lot**	**amount**
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM083 TAE, 10X	_____	_____	_____
S001 20% SDS	_____	_____	_____

Initials: RCJ Date: 4/14/92

made by: _____ date: _____

Initials: RCF Date: 4/14/92

S021 YIELD GEL LOADING BUFFER lot number: _____

standard batch size: 100 ml

INGREDIENTS	final concentration	amount
RM020 bromophenol blue	0.25%	0.25 ± 0.01 g
RM217 xylene cyanol	0.25%	0.25 ± 0.01 g
RM040 ficoll 400	12.5%	12.5 ± 0.1 g
S009 EDTA, 0.5M	50. mM	10.0 ± 0.1 ml
RM083 TAE, 10X	5.0 X	50.0 ± 0.5 ml
S001 SDS, 20%	0.20 %	1.00 ± 0.02 mL

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	**source**	**lot**	**amount**
RM020 bromophenol blue	_____	_____	_____
RM217 xylene cyanol	_____	_____	_____
RM040 ficoll 400	_____	_____	_____
S009 EDTA, 0.5M	_____	_____	_____
RM083 TAE, 10X	_____	_____	_____
S001 20% SDS	_____	_____	_____

Initials: RCJ Date: 4/14/92

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S032 LAMBDA HIND III, 20NG/μL lot number: _____

INGREDIENTS	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM155 lambda Hind III fragments			20 ng/μl	
S021 yield gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

CALCULATIONS

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

lculate the final volume according to equation 1.

$$(\text{final volume}) = \frac{(\text{initial DNA concentration})(\text{initial DNA volume})}{(20\ \text{ng}/\mu\text{l})} \quad \text{equation 1}$$

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.

$$(\text{buffer volume}) = 0.2(\text{final volume}) \quad \text{equation 2}$$

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

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

Record the buffer and water volumes above.

Initials: RCJ Date: 4/14/92

LAMBDA HIND III, 20NG/µL lot number: _____

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.**S032**

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	**source**	**lot**	**amount**
M155 lambda Hind III fragments	_____	_____	_____
S021 yield gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

made by: _____ date: _____

Initials: RCS Date: 4/14/92

S036 TRIS-HCl, 1M - PH 7.4 lot number: _____

standard batch size: 250 ml

INGREDIENTS	final concentration	amount
RM073 TRIS	1.00 M	30.3 ± 0.1 g
RM096 hydrochloric acid	------	------

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	**source**	**lot**	**amount**
RM073 TRIS	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

final pH: _____ specification: 7.4 ± 0.1

1:100 pH: _____ specification: 7.4 ± 0.1

Initials: RCS Date: 4/14/92

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S042 PHI-X MARKER lot number: _____

INGREDIENTS	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM156 phi-X-174, Hae III fragments			50 ng/μl	
S018 analytical gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

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.

lculate the final volume according to equation 1.

$$\text{(final volume)} = \frac{\text{(initial DNA concentration)(initial DNA volume)}}{\text{(50 ng/}\mu\text{l)}} \quad \text{equation 1}$$

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.

$$\text{(buffer volume)} = 0.2\text{(final volume)} \quad \text{equation 2}$$

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

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

Record the buffer and water volumes above.

Initials: RCJ Date: 4/14/92

S042 PHI-X MARKER lot number: _____

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.1

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	**source**	**lot**	**amount**
M156 phi-X-174 Hae III fragments	_____	_____	_____
S018 analytical gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

made by: _____ date: _____

Initials: LCJ Date: 4/14/92

S060 CALIBRATION CONTROL lot number: _____

INGREDIENTS	initial concentration (ng/μl)	initial volume (μl)	final concentration	final volume (μl)
RM221 K562 DNA			5 ng/μl	
S021 yield gel loading buffer	5 X		1 X	----
S059 sterile water	-----		----	----

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.

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

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.

$$\text{(buffer volume)} = 0.2\text{(final volume)} \quad \text{equation 2}$$

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

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

Record the buffer and water volumes above.

Initials: RCJ Date: 4/14/92

S060 CALIBRATION CONTROL lot number: _____

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.

DATA LOG	**source**	**lot**	**amount**
RM221 K562 DNA	_____	_____	_____
S021 yield gel loading buffer	_____	_____	_____
S059 sterile water	_____	_____	_____

made by: _____ date: _____

Initials: RCJ Date: 4/14/92

S064 CELL PELLET CONTROL lot number: _____

INGREDIENTS	concentration of cells	total volume (ml)	cells per aliquot	aliquot volume (ml)
RM243 K562 cells			$1 \cdot 10^6$	
S034 phosphate buffered saline (PBS)	----	----	----	----

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 $1 \cdot 10^6$ cells according to equation 1.

$$\text{(aliquot volume)} = \frac{(1 \cdot 10^6 \text{cells})}{\text{(concentration of cells)}} \qquad \text{equation 1}$$

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.

Initials: RC Date: 4/14/92

S064 CELL PELLET CONTROL lot number: _____

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	**source**	**lot**	**amount**
RM243 K562 cells	_____	_____	_____
S034 phosphate buffered saline	_____	_____	_____

made by: _____ date: _____

Initials: JCJ Date: 3/10/93

S034 Phosphate Buffered Saline (PBS) lot number: _____

Standard batch size: 4 L

Ingredients	final concentration	amount
RM005 sodium chloride	137 mM	32.0 ± 0.1 g
RM053 potassium chloride	3.0 mM	0.90 ± 0.01 g
RM065 sodium phosphate, dibasic	6.0 mM	3.41 ± 0.03 g
RM056 potassium phosphate, monobasic	1.5 mM	0.82 ± 0.02 g

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	source	lot	amount
RM005 sodium chloride	_____	_____	_____
RM053 potassium chloride	_____	_____	_____
RM065 sodium phosphate, dibasic	_____	_____	_____
RM056 potassium phosphate, monobasic	_____	_____	_____

Quality Control

final pH: _____ spec: 7.5 ± 0.1

1004 DQα differential extraction

made by: _____ date: _____

Initials: RCJ Date: 3/10/93

S082 Chelex, 20% lot number: _____

Standard batch size: 500 ml

Ingredients	final concentration	amount
RM027 chelex 100	20. %	100 ± 2 g
S059 sterile water	---	450 ± 50 ml (guideline)

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	source	lot	amount
RM027 chelex 100	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QC004 DQα differential extraction

made by: _____ date: _____

Initials: RCJ Date: 3/10/93

S093 DTT, 1M lot number: _____

Standard batch size: 20 ml

Ingredients	final concentration	amount
RM101 dithiothreitol	1.0 M	3.1 ± 0.2 g
S059 sterile water	-----	-----

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	source	lot	amount
RM101 dithiothreitol	_____	_____	_____
S059 sterile water	_____	_____	_____

Quality Control

QA004 DQα differential extraction

made by: _____ date: _____

Initials: RCS Date: 3/10/93

S094 Digest Buffer lot number: _____

Standard batch size: 6 L

Ingredients	final concentration	amount
S009 EDTA, 0.5M	10. mM	120 ± 6 ml
S036 TRIS-HCl, 1M-pH 7.4	10. mM	60 ± 3 ml
S012 sodium chloride, 5M	50. mM	60 ± 1 ml
S001 SDS, 20%	2.0 %	600 ± 15 ml
RM096 hydrochloric acid	---	---

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.

Mix well.

Measure and record the final pH.

Aliquot into 50 ml centrifuge tubes.

Store at room temperature.

Data Log	source	lot	amount
S009 EDTA, 0.5M	_____	_____	_____
S036 TRIS-HCl, 1M-pH 7.4	_____	_____	_____
S012 sodium chloride 5M	_____	_____	_____
S001 SDS, 20%	_____	_____	_____
RM096 hydrochloric acid	_____	_____	_____

Quality Control

final pH: _____ specification: 7.5 ± 0.1

S004 differential extraction

made by: _____ date: _____