





Appendix B

Prepared Buffers and Solutions



DNA Unit DNA Database Unit





0.5M EDTA

I. Required Items:

NaOH Pellets

Total volume 1 L 500 ml 100ml

II. <u>Instructions For Preparation</u>:

Add EDTA to dH_2O . Add NaOH pellets to get EDTA into solution. Adjust volume to 1L. Calibrate pH meter with pH 7.0 standard buffer. Analyst initials preparing the buffer indicate that the calibration check was done. Adjust pH to 8.0 with NaOH pellets. Filter sterilize.

III. Storage Conditions:

Refrigerate at 4°C.

IV. Expiration Date:

6 months

Vendor/Lot # EDTA	Vendor/Lot # NaOH	Amt. Made	Date Prepared	Analyst	Expiration Date
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<u> 10N NaOH</u>

I. Required Items:

 $\begin{array}{ccc} NaOH & 40~g & 80~g \\ dH_2O & 70~ml & 140~ml \end{array}$

Total volume 100 ml 200ml

II. <u>Instructions For Preparation</u>:

Add NaOH to dH₂O and mix well. Bring to final volume of 100 ml. CAUTION: This will generate heat.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Prep Date NaOH	Amt. Made	Date Prepared	Analyst	Expiration Date

20% SDS

I. Required Items:

 $\begin{array}{ccc} \text{Sodium dodecyl sulfate} & 200 \text{ g} & & 20 \text{ g} \\ \text{dH}_2\text{O} & 800 \text{ ml} & & 80 \text{ ml} \end{array}$

Total volume 1 L 100 ml

II. Instructions For Preparation:

Dissolve sodium dodecyl sulfate in dH₂O. To aid dissolution, solution may be heated. Adjust to volume with dH₂O.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Vendor/Lot # SDS	Amt. Made	Date Prepared	Analyst	Expiration Date

Stain Extraction Buffer

I. Required Items:

 $\begin{array}{lll} {\rm Tris} & & 1.21 \ g \\ {\rm Na}_2 \ {\rm EDTA} - 2 \ {\rm H}_2{\rm 0} & & 3.7 \ g \\ {\rm NaCl} & & 5.84 \ g \\ {\rm 20\% \ SDS} & & 100 \ ml \end{array}$

 dH_2O 500 * see below

Total volume 1 L

11. Insuluctions for lieparation.

Add NaCl, EDTA, and Tris to 500 ml of dH_2O . Calibrate pH meter with pH 7.0 standard buffer. Analyst initials preparing the buffer indicate that the calibration check was done. Titrate to pH8.0 with NaOH. (DO FIRST). Then add the SDS. Then bring the final volume to 1 L with dH_2O . Autoclave.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Expires 3 months after preparation or 6 months after preparation if unopened..

Vendor/Lot # NaCl	Vendor/Lot # Tris	Vendor/Lot # Na ₂ EDTA - 2 H ₂ 0	Date SDS Made	Amt. Made	Date Prepared	Analyst	Expiration Date
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STR 10X Borate Tris Buffer (Stock Solution)

I. Required Items:

Boric Acid	55.6 g	111.2 g		
dH_2O	700 ml	1400 ml		
Total volume	1 L	2 L		

II. <u>Instructions For Preparation</u>:

Dissolve acid in dH_2O while stirring. Bring to final volume with dH_2O . Dilute 1:20 with dH_2O for tank buffer.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Vendor/Lot # Boric acid	Vendor/ Lot# Tris	Amt. Made	Date Prepared	Analyst	Expiration Date
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STR .39M **DTT**

I. Required Items:

 $\begin{array}{ll} Dithiothreitol & 601 \ mg \\ sterile \ dH_2O & 10 \ ml \end{array}$

Total volume 10 ml

II. <u>Instructions For Preparation</u>:

Add DTT to water and mix well. Aliquot 200µl into sterile .5 ml tubes.

III. Storage Conditions:

Freeze at -20 °C.

IV. Expiration Date:

Vendor/Lot # DTT	Amt. Made	Date Prepared	Analyst	Expiration Date

STR Citrate Buffer

(.1M Sodium Citrate, pH 5.0)

I. Required Items:

Trisodium citrate, dihydrate ($Na_3C_6H_5O_7*2H_2O$)

18.4g dH_2O 800 ml

Citric acid monohydrate

70 tal volume

1 Liter

II. Instructions For Preparation:

Add Trisodium citrate to water. Calibrate pH meter with pH 7.0 standard buffer. Analyst initials preparing the buffer indicate that the calibration check was done. pH to 5.0 with approximately 6 g of citric acid monohydrate. Adjust volume to 1 Liter.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Lot# Trisodium	Lot# Citric Acid	Amt.	Date	Analyst	Expiration
Citrate	monohydrate	Made	Prepared		Date

STR Hybridization Solution (5X SSPE. .5% w/v SDS)

I. Required Items:

20X SSPE 250 ml 20% w/v SDS 25 ml dH_2O 725 ml

Total volume 1 Liter

II. <u>Instructions For Preparation</u>:

Add SSPE and SDS to water and mix well. Solids must be in solution before use.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Prep Date SSPE	Prep Date SDS	Amt. Made	Date Prepared	Analyst	Expiration Date

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STR Prewetting Solution

(.4N NaOH, 25 mM EDTA)

I. Required Items:

 $\begin{array}{ccc} 10N \ NaOH & 20 \ ml \\ .5M \ EDTA & 25 \ ml \\ dH_2O & 455 \ ml \end{array}$

Total volume 500 ml

II. <u>Instructions For Preparation</u>:

Add ingredients and mix well.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Prep Date NaOH	Prep Date EDTA	Amt. Made	Date Prepared	Analyst	Expiration Date

STR Proteinase K

I. Required Items:

 $\begin{array}{ll} Proteinase \ K & 100 \ mg \\ sterile \ dH_2O & 10 \ ml \end{array}$

Total volume 10 ml

II. <u>Instructions For Preparation</u>:

Add Proteinase K to water and mix well. Aliquot 100 μl into sterile .5 ml tubes.

III. Storage Conditions:

Freeze at -20 °C. **DO NOT RE-FREEZE ALIQUOTS.**

IV. Expiration Date:

Vendor/Lot # Proteinase K	Amt. Made	Date Prepared	Analyst	Expiration Date

STR Spotting Solution (.4N NaOH, 25 mM EDTA, .00008% Bromothymol Blue)

I. Required Items:

5N NaOH	6ml	12 ml
.5M EDTA	3.75 ml	7.5 ml
Bromothymol blue	150 μ1	300 µ1
dH_2O	65 ml	

Total volume 75 ml 150 ml

II. <u>Instructions For Preparation</u>:

Add ingredients and mix thoroughly.

III. Storage Conditions:

IV. Expiration Date:

Expires 3 months after preparation.

Prep Date NaOH	Prep Date EDTA	Amt. Made	Date Prepared	Analyst	Expiration Date

STR 20X SSPE
(3.6M NaCl, 200 mM Sodium Phosphate, 20 mM EDTA, pH7.4)

I. Required Items:

Disodium EDTA	7.4 g	14.8 g
NaCl	210 g	420 g
Sodium Phosphate, monobasic	27.6 g	55.2 g
10 N NaOH	~10 ml	~20 ml
dH_2O	800 ml	1100 ml
Total volume	1 L	2 L

II. <u>Instructions For Preparation</u>:

Add EDTA to 800 ml DH₂O. Calibrate pH meter with pH 7.0 standard buffer. Analyst initials preparing the buffer

indicate that the calibration check was done. Adjust pH to 8.0 with 10N NaOH. Add NaCl and Sodium Phosphate and pH to 7.4 with ~ 10 ml of 10N NaOH. Adjust final volume to 1 L.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Expires 3 months after preparation.

Lot # EDTA	Lot # Sodium Phosphate	Sate NaOH Made	Lot # NaCl	Amt. Made	Date Prepared	Analyst	Expiration Date

STR TE -4

I. Required Items:

 $\begin{array}{ll} \text{1M Tris HCl} & \text{10 ml} \\ \text{.5M EDTA} & \text{200 } \mu \text{l} \\ \text{dH}_2\text{O} & \text{990 ml} \end{array}$

Total volume 1 L

II. Instructions For Preparation:

Add Tris HCl and EDTA to dH₂O. Autoclave.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

DateTris HCl Made	Date EDTA Made	Amt. Made	Date Prepared	Analyst	Expiration Date

STR Tris/EDTA/NaCl

(pH 8.0)

I. Required Items:

NaCl	.584g	1.17 g
1M Tris-HCl	1 ml	2 ml
.5M EDTA	200 μ1	400 μ1
dH_2O	~70 ml	~140 ml

Total volume 100 ml 200 ml

II. <u>Instructions For Preparation</u>:

Add NaCl, EDTA, and Tris-HCl to dH_2O and stir until dissolved. Calibrate pH meter with pH 7.0 standard buffer. Analyst initials preparing the buffer indicate that the calibration check was done. Adjust to pH 8.0 with 1.0 N HCl. Bring to final volume with dH_2O . Autoclave.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Vendor/Lot # NaCl	Date Tris-HCl Made	Date EDTA Made	Amt. Made	Date Prepared	Analyst	Expiration Date

STR Wash Solution (1.5X SSPE, .5% w/v SDS)

I. Required Items:

20X SSPE 150 ml 300 ml 20% w/v SDS 50 ml 100 ml dH_2O 1800 ml 3600 ml

Total volume 2 Liters 4 Liters

II. <u>Instructions For Preparation</u>:

Add SSPE and SDS to water and mix well.

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

1 M Tris-HCL

I. Required Items

 $\begin{array}{cc} \text{Tris base} & 121.2 \text{ g} \\ \text{d H}_2\text{O} & 800 \text{ ml} \end{array}$

conc. HCl

Final Volume 1000 ml

II. Instructions for Preparation

Add Tris and dH_2O . Calibrate pH meter with pH 7.0 standard buffer. Analyst initials preparing the buffer indicate that the calibration check was done. Adjust pH to 8.0 (\pm 0.2) by adding ~45 ml of conc. HCl. Bring to final volume with dH_2O . Autoclave.

III. Storage Conditions:

Room temperature

IV. Expiration Date:

Vendor/Lot # Tris	Vendor/Lot # HCl	Amt. Made	Date Prepared	Analyst	Expiration Date

5% Long Ranger Polyacrylimide Stock Solution

CAUTION - ACRYLAMIDE IS A SUSPECTED NEUROTOXIN - WEAR GLOVES AND EYE PROTECTION***

I. Required Items:

Urea	18.0 g	360 g
d H ₂ O	26.0 ml	520 ml
10X TBE	5.0 ml	100 ml
50% Long Ranger gel solution	5.0 ml	100 ml
Final volume	50 ml	1.0 L

II. <u>Instructions for Preparation:</u>

Mix and dissolve all components thoroughly. **Long Ranger Stock <u>must be filtered before use.</u>**

III. Storage Conditions:

Stock must be kept in a light sensitive bottle at 4° C.

IV. Expiration Date:

Vendor/Lot # Urea	Vendor/Lot # 10X TBE	Vendor/Lot# 50% Long Ranger	Amount Made	Date Prepared	Analyst	Expiration Date

Qiagen AW1 Buffer

I. Required Items:

AW1 concentrate supplied from Qiagen extraction kit and 100% pure ethanol.

II. Instructions For Preparation:

KIT SIZE	AW1 CONCENTRATE	ETHANOL	FINAL VOLUME
50	19 ml	25 ml	44 ml
250	95 ml	125 ml	220 ml
1000	175 ml	230 ml	405 ml

III. Storage Conditions:

Store closed at room temperature.

IV. Expiration Date:

Expires 3 months once opened.

Vendor/Lot#	Date Opened	Analyst	Expiration Date
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2M NaOH

I. Required Items:

 $\begin{array}{ccc} NaOH & 40~g & 80~g \\ dH_2O & 450~ml & 900~ml \end{array}$

Total volume 500 ml 1 liter

II. <u>Instructions For Preparation</u>:

Add NaOH to dH₂O and mix well. Bring to final volume. CAUTION: This will generate heat.

10 M NaOH may be diluted (200 ml NaOH to 800 ml dH_20) to prepare 2 M NaOH

III. Storage Conditions:

Room Temperature

IV. Expiration Date:

Prep Date NaOH	Amt. Made	Date Prepared	Analyst	Expiration Date