

Procedure 6.3.1 – Reagent Preparation – cDNA Solutions

Date: \_\_\_\_\_

Experiment ID: 6.3.1-*yyyyyuuuuuu*

Technician: \_\_\_\_\_

Project: \_\_\_\_\_

Client Signature: \_\_\_\_\_

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➤ Low-dCTP dNTP Solution

Combine the following (Invitrogen 100mM dNTP set). \_\_\_\_\_

dGTP (100mM)	25 ul	_____
dATP (100mM)	25 ul	_____
dTTP (100mM)	25 ul	_____
dCTP (100mM)	10 ul	_____
Nuclease-free water	415ul	_____

Enter into the LMT reagent logbook (Appendix 7.3). \_\_\_\_\_

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➤ Prehybridization Solution

Combine the following.

Deionized Formamide	5000 ul	_____
20X SSPE	3000 ul	_____
10% SDS	500 ul	_____
50X Denhardt's	1000 ul	_____
Salmon Sperm DNA (10mg/ml)	200 ul	_____
Deionized Water	930 ul	_____

Divide solution into 400ul aliquots and store at -20C in the *Prehybridization Solution* box.

➤ **Hybridization Solution**

Combine the following.

Deionized Formamide	5000 ul	_____
10% SDS	360 ul	_____
50X Denhardt's	710 ul	_____
20X SSPE	2860 ul	_____

Divide solution into 400ul aliquots and store at -20C in the *Hybridization Solution* box.

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➤ **20X Blocking Solution**

1. Combine the following in a 15ml conical tube.

Poly-dA (1ug/ul)	185 ul	_____
tRNA (10ug/ul)	37 ul	_____
Appropriate Cot DNA (1ug/ul)	925 ul	_____
Sodium Acetate (3M)	130 ul	_____
Ethanol (100%)	3240 ul	_____

2. Vortex and divide into 5 micro centrifuge tubes (900ul each).

3. Incubate at -20C for one hour.

4. Centrifuge at maximum speed for 15 minutes at 4C.

5. Wash pellet with 75% ice-cold ethanol by centrifuging at maximum speed for 15 minutes at 4C.

6. Remove residual EtOH with a pipettor.

7. Resuspend the pellet in 20ul nuclease-free water. \_\_\_\_\_

8. Enter into LMT Reagent LogBook. \_\_\_\_\_

9. Store at -20C in *20X Blocking Reagents* box.

➤ **Wash Solutions**

**Wash Solution 1**      **(1X SSC, 0.1% SDS)**

**20X SSC**      **50 ml**  
**10% SDS**      **10 ml**  
**DI Water**      **940 ml**

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**Wash Solution 2**      **(0.2X SSC, 0.1% SDS)**

**20X SSC**      **10 ml**  
**10% SDS**      **10 ml**  
**DI Water**      **980 ml**

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**Wash Solution 3**      **(0.2X SSC)**

**20X SSC**      **10 ml**  
**DI Water**      **990 ml**

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**Wash Solution 4**      **(0.1X SSC)**

**20X SSC**      **5 ml**  
**DI Water**      **990 ml**

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