

IN SITU HYBRIDIZATION PROTOCOL
STOCK SOLUTIONS

I. HYBRIZATION STOCK SOLUTION:

◆ 50% Deionized Formamide	50 ml
◆ 5X Denhards solution	10 ml of 50X solution (or 5ml of a 100X)
◆ 10% Dextran sulphate	10 grams
◆ 0.5% Sodium pyrophosphate	0.5 grams
◆ 0.5% SDS	5 ml of 10 %
◆ DEPC H ₂ O to 100 ml final	35 ml

1. Put first 3 ingredients on stirrer and dissolve.
2. Once all the 3 substances are dissolved, continue adding the last ingredients.
3. Apply some heat but do not exceed 40°C.
4. Aliquot in 1 ml tubes. Store at -20 °C.

II. PHOSPHATE BUFFERED SALINE (PBS):

	<u>2000 ml</u>
◆ NaCl	17.58 grams
◆ KH ₂ PO ₄	0.544 grams
◆ Na ₂ HPO ₄ (anhydrous)	2.27 grams

NOTE: Makes up to 2 litres using double distilled water (dd H₂O)

1. Add above reagents to 2 litres of ddH₂O and stir the solution on a hot plate at room temperature.
2. In order to insure that the solution is RNase-free, add 2 ml of DEPC to 2000 ml of PBS. Add the DEPC in the fumehood and stir the solution for a minimum of 2 hours.
3. Pour the PBS/DEPC solution into the PBS labelled bottles and autoclave the bottles for 25 minutes. (The solution is autoclaved in the bottle in order to destroy the DEPC).
4. Allow the bottles to cool before adding the solution to the PBS tank.

III. DEPC-TREATED WATER (DEPC H₂O):

1. Add 2 ml of DEPC to every 2000 ml of double distilled water (dd H₂O) and let the solution stir for a minimum of 2 hours in the fumehood.
2. Pour the solution in DEPC labelled bottles and autoclave the bottles for 25 minutes (The solution is autoclaved in order to destroy the DEPC).

IV. 20x SSC in DEPC-TREATED WATER:

	<u>1000 ml</u>	<u>2000 ml</u>
◆ NaCl	175.3 grams	350.6 grams
◆ Sodium Citrate	88.2 grams	176.4 grams

1. Add 800 or 1600 ml DEPC-treated water to large Erlenmeyer flask on stirrer.
2. Add the salts to the solution and allow them to dissolve.
3. Adjust pH to 7.0 with 10 and 1M HCl acid.
4. Adjust the volume to 1 or 2 litres with DEPC-treated water.
5. Autoclave for 25 minutes on slow setting.

V. 4M NaCl in DEPC-TREATED WATER:

	<u>1000 ml</u>
NaCl	233.76 grams

1. Add water to NaCl to make up 1000 ml and stir until NaCl dissolves.
2. Autoclave for 25 minutes on slow setting.