

IN SITU HYBRIDIZATION PROTOCOL **PREHYBRIDIZATION AND HYBRIDIZATION**

A slide holder carries approximately 24 slides which requires approximately 300 ml of solution to cover it in the glass basins.

1. Take the slides from -80°C freezer just before use, thaw and check that they have the required tissue on them.
2. Place the slides in the following solutions for the time given below. All incubations are performed at **room temperature** unless otherwise stated:

<u>Solution</u>	<u>Time</u>
◆ PBS	5 minutes
◆ 0.1M Glycine	5 minutes
◆ PBS	5 minutes
◆ 0.3% Triton	5 minutes (at this point, take protease K from freezer)
◆ PBS	3 minutes
◆ PBS	3 minutes
◆ Proteinase K	20 minutes exact - at 37°C (in incubator)
◆ 4% Paraformaldehyde	5 minutes RT exact
◆ PBS	5 minutes RT
◆ PBS	5 minutes RT
◆ Autoradiography solution (1)	10 minutes -exact- at 37°C (in incubator)
◆ Autoradiography solution (2)	20 minutes -exact- at 37°C (in incubator)
◆ 50% Formamide in 2X SSC	15 minutes at 37°C (in incubator)
◆ 70% Ethanol	5 minutes
◆ 90% Ethanol	5 minutes
◆ 100% Ethanol	10 minutes
◆ Dry slides in fumehood for about an hour.	

Hybridization mixture.

3. Warm hybridization buffer in waterbath at 60°C and vortex.
4. Label tubes and take radioactive probe from -80°C freezer.
5. Make hybridization mixture according to the following instructions:

Example

Total volume needed per slide is 15 µl

Radioactivity required is usually 0.75×10^6 per slide

NOTE: Always prepare enough solution and radioactivity for 1 extra slide to make up for losses.

for example, we want to do in situ hybridization on 9 slides:

- we have 9 slides + 1 extra slide = 10 slides
- the probe concentration (for this example) is $0.9 \times 10^6/\mu\text{l}$ (as given by counts determined following labelling)
- therefore we need: $10 \times (0.75 \times 10^6) = 7.5 \times 10^6$ CPM/ 150 µl total volume
- hybridization buffer is always 90% of the total volume.
- water is just to make up the final volume.

As such, we use:

Hybridization Buffer	135 μ l
Probe volume	8.3 μ l
DTT(stocksol. 5M)	1.5 μ l (50 mM final)
DEPC water	5.2 μ l

6. Vortex the mixture and then centrifuge it up to 10 000 RPM.
7. Heat the probe mixture in a sterile microfuge tube in the waterbath at 60°C shaking at 130 strokes for 30 minutes (since heating to denature the probe is not always recommended, check to see if this should be done.
8. Cool the probe mixture on ice for 5 minutes.
9. Reheat the probe mixture to 40 °C in incubator for 10 minutes maximum.
10. In the meantime, prepare the slides by labelling them with a pencil and use a diamond marker to score on the back of the slide circling the tissue.
11. Prepare humid boxes by placing a sheet of paper towel on the bottom and about 1 cm of 4X SSC on the base.
12. Add probe to slides (15 μ l per slide), making sure there are no air bubbles (vortex the tube in order to remove any.)
13. Put round cover slips in place and transfer box to incubator. The temperature of the incubator should be set on 40-42 °C
14. Prepare the solutions for post-hybridization phase.