

Riboprobe Labeling

Template

H ₂ O		_____	_____	_____	_____
100mM DTT	2	µl			
5 X transcription buffer	4	µl			
RNase inhibitor	0.5	µl			
10mM A,G,C	2	µl			
10mM UTP	1.3	µl			
10mM DIG-UTP	0.7	µl			
template-DNA (1µg)			_____	_____	_____
RNA polymerase	2	µl			
total					20 µl

37°C 2 hr _____ : _____ - _____ : _____

0.5 M EDTA 0.8 µl

8M LiCl 1 µ

ethanol 60 µl

-20°C O/N _____ / _____ / _____ : _____ am/pm

probe alkali hydrolysis

t = $(L_o - L_f) / k L_o L_f$ _____ min _____ min _____ min

_____ min

H₂O 49µl

5N NaOH 1µl

_____ : _____ : _____ : _____

neutralization

0.1N HCl 45µl

1M tris (pH7.5) 5µl

_____ : _____ : _____ : _____

Section preparation

PBS R/T	3min	_____
0.3% Triton X-100 in PBS		
R/T	10min	_____
PBS R/T	3min	_____
PBS R/T	3min	_____
proteinase K (1µg/ml in 100mM tris, 50mM EDTA)		
37°C	20min	_____
4% paraform-aldehyde in PBS		
R/T	3min	_____
PBS R/T	3min	_____
Acetylation		
R/T	10min	_____

Section preparation for fresh frozen section

4%

paraform-aldehyde in PBS	5min	
PBS	3min	
PBS	3min	
PBS	3min	
Acetylation	10min	
<i>prehybridization</i>		
R/T	2hr	_____ : _____ - _____ : _____

Hybridization

42°C 12-16 hr. _____ : _____ - _____ : _____

Washing

5min X SSC 50% Formamide °C 20min RNase-buffer rinse
RNase treatment 37°C 30min RNase-buffer 37°C
30min X SSC 50% Formamide °C 12min
X SSC 50% Formamide °C 12min 2X SSC R/T 5min
2X SSC R/T 5min 0.1M Tris 0.15M NaCl R/T
5min

detection

Immunological
Blocking : -

: Anti-DIG / / :

Anti-DIG Washing

Buffer1 10 min

Buffer1 10 min

Buffer1 10 min

Buffer2 10 min

Start color reaction / / :

exchange color solution / / :

/ / :

/ / :

/ / :

/ / :

Stop color reaction with buffer 3.