

Digoxigenin labelled riboprobe synthesis

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RNase-free solutions needed:

10X transcription buffer*

0.4 M Tris pH 7.5

60 mM MgCl₂

100 mM NaCl

20 mM spermidine-HCl

10X dig U NTP mix*

10 mM ATP

10 mM GTP

10 mM CTP

6 mM UTP

4 mM dig-UTP

*These solutions can be purchased ready made from Boehringer Mannheim

2X carbonate buffer

120 mM Na₂CO₃

80 mM NaHCO₃

pH to 10.2

Aliquot and store at -20°C

Stop solution

0.2 M NaAc

pH to 6.0 with HOAc

50 mM DTT

DEPC-treated deionized water

20 mgs/ml glycogen

4M LiCl

EtOH

Also needed:

T3, T7, or SP6 RNA polymerase

RNasin (optional)

RNase-free DNase I

proteinase K

phenol: chloroform

Procedure

1) Linearize 5-10 ugs of template DNA completely with an enzyme which cuts at the 5' end of the insert in PBS and which **does not** leave a 3' overhang.

2) Add proteinase K to 200 ug/ml and incubate at 37°C for 30 min.

3) Add 1/4 volume of 10 M NH₄Ac.

4) Extract with an equal volume of phenol:chloroform:isoamyl alcohol.

From this point on, handle the DNA with RNase-free tips and use RNase-free reagents.

5) EtOH precipitate with 2 volumes of EtOH. Spin for 10 min. Rinse pellet with 70% EtOH. Air dry.

6) Resuspend DNA in 20 ul diw. Quantitate on a slide gel or on a spec.

7) Combine:

1 ug DNA (in less than 4 ul diw)
2 ul 10X transcription buffer
2 ul 10X dig mix
2 ul 50 mM DTT
2 ul RNasin (optional)
diw to 18 ul

2 ul T3, T7, or SP6 polymerase
20 ul

Incubate at 37°C for 2 hrs.

8) Destroy template by adding 2 ul of DNase I and incubating at 37°C for 15 min.

9) Hydrolyze probe by adding 20 ul of diw and 40 ul of carbonate buffer and incubating at 65°C for 40 min.

10) Neutralize with 80 ul of stop solution.

11) Add 16 ul of 4 M LiCl; 10 ul of glycogen, and 400 ul of EtOH. Mix and freeze at -80°C for at least 10 min.

12) Spin hard for 15 min. Rinse pellets with 70% EtOH.

13) Resuspend probes in 100 ul of *in situ* hyb buffer. Quantitate by dot blot using digoxigenin labelled RNA standards from BMB. Store probes at -20°C or in small aliquots at -80°C.

We typically use these probes at 0.5-1 ul/100 ul hybridization.