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In vitro transcription of DIG-labelled RNA probe

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Ida Barlow[1]

[1]Imperial College London

Ida Barlow

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Protocol status: Working
We use this protocol and it's working

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Abstract

Protocol for making DIG-labelled RNA probes suitable for *in situ* hybridisation

Materials

MATERIALS

- DTT **Merck MilliporeSigma (Sigma-Aldrich) Catalog #**D0632
- T3 RNA Polymerase, 1,000u **Promega Catalog #**P2083
- DNaseI **NEB**
- RNA clean & concentrator-25 **Zymo Research Catalog #**R1017
- RNase Inhibitor **Thermo Fisher Catalog #**N8080119
- Digoxigenin-11-UTP **Merck Millipore (EMD Millipore) Catalog #**11209256910

1 Assemble reaction on ice:

2 µL DTT

2 µL 10X DIG-NTP mix (5 mM)

0.5 µL RNAse inhibitor

2 µL 10 X transcription buffer

1 µg linearlised template

1 µL RNA polymerase

make up to 20ul total volume with WATER

20 µL TOTAL volume

Note

5mM DIG-NTP mix (10X):

2 µL 100mM CTP

2 µL 100mM GTP

2 µL 100mM ATP

1.3 µL 100mM UTP

7 µL 10mM DIG-11-UTP

25.7 µL Nuclease-free water

2 Mix well by gently flicking and spin down tube contents

3 Incubate at 37 °C for 02:00:00

4 Add 1 µL DNaseI and digest the template at 37 °C for 00:15:00

5 Use ZYMO RNA cleanup kit to purify RNA and check quality by agarose gel electrophoresis; quantiy with nanodrop spectrophotometer