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In vitro transcription of DIG-labelled RNA probe

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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 linearised 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