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In vitro transcription of DIG-labelled RNA probe

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1 Works for me dx.doi.org/10.17504/protocols.io.ba4pign

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ABSTRACT

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

MATERIALS

| NAME ▾ | CATALOG # ▾ | VENDOR ▾ |
|-----------------------------|-------------|-----------------|
| DTT | D0632 | Sigma Aldrich |
| T3 RNA Polymerase, 1,000u | P2083 | Promega |
| DNaseI | | NEB |
| RNA clean & concentrator-25 | R1017 | Zymo Research |
| RNase Inhibitor | N8080119 | Thermo Fisher |
| Digoxigenin-11-UTP | 11209256910 | Merck Millipore |

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



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



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