JAN 14, 2020

In vitro transcription of DIG-labelled RNA probe

Ida Barlow¹

¹Imperial College London

Ida Barlow

ABSTRACT

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

MATERIALS

MATERIALS

DTT **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 Catalog #11209256910**

OPEN ACCESS

DOI:

dx.doi.org/10.17504/protocols.io.ba4pigvn

Protocol Citation: Ida Barlow 2020. *In vitro* transcription of DIG-labelled RNA probe.

protocols.io

<https://dx.doi.org/10.17504/protocols.io.ba4pigvn>

License: This is an open access protocol distributed under the terms of the [Creative Commons Attribution License](#), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: Jan 08, 2020

Last Modified: Jan 14, 2020

PROTOCOL integer ID:

31599

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