In vitro transcription of DIG-labelled RNA probe

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Jan 14, 2020

1 Works for me

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

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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 linearlised 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