Radioactive in vitro transcription

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Abstract

Protocol for radioactive labelling of RNA using T7-Polymerase and α-[^{32}P]-UTP.

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Protocol

Buffers and reagents required

Step 1.

- 5x Ribomax buffer: 400 mM Hepes-KOH, pH 7.5; 60 mM $MgCl_2$; 10 mM spermidine; 200 mM DTT

Before start:

Step 2.

Generate a PCR product using primers containing the T7-promoter. Gel-extract or clean up using column purification.

Pipetting scheme

Step 3.

PCR Product containing the T7-Promoter (100 ng - 1 µg)

Ribomax buffer	6 µL
Pyrophosphatase	1 µL
RNase inhibitor	0.5 µL
DTT (1 M)	2 µL
ATP 10 mM	1.8 µL
CTP 10 mM	1.8 µL
GTP 10 mM	1.8 µL
α-[^{32}P]-UTP	3 µL

T7 RNA-Polymerase	1 µL
H_2O *ad*	30 µL

Incubate reaction for at least 2 hours at 37˚C.

DURATION
02:00:00

Clean-up

Step 4.

Remove excessive radioactive nucleotides by cleaning the probe using a G-50 column (GE-Healthcare).