Protocol

Conventional *in vitro* Transcription

This protocol is for the Conventional *in vitro* Transcription

More than 10 µg of RNA transcript can be generated per 1 µg template DNA using the following protocol. The reaction can be scaled up or down. For high yield transcription, generating up to 200 µg RNA, use TranscriptAid™ T7 High Yield Transcription Kit.

- Thaw frozen reagents, mix and centrifuge briefly.
- Keep enzymes and nucleotides on ice.
- Keep the Reaction Buffer at room temperature.

1. Prepare the following reaction mixture at room temperature:

5X Transcription buffer	10 µl
ATP/GTP/CTP/UTP Mix, 10 mM each	10 µl (2 mM final concentration)
Linearized template DNA	1 µg
RiboLock™ RNase Inhibitor	1.25 µl (50 u)
T7/T3/SP6 RNA Polymerase	1.5 µl (30 u)
DEPC-treated Water	to 50 µl
Total volume	**50 µl**

2. Incubate at 37°C for 2 hours.
3. Optional: To remove template DNA add 2 µl (2 u) of DNase I, RNase-free, mix and incubate at 37°C for 15 min.
4. Stop the reaction by addition of 2 µl 0.5 M EDTA, pH 8.0 and incubate at 65°C for 10 min.

Note
RNA hydrolyzes if heated in the absence of a chelating agent.

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North America
Technical Services:
techservice.genomics@thermofisher.com
Customer Services:
customerservice.genomics@thermofisher.com
Tel 800 235 9880
Fax 800 292 6088

Europe and Asia
Technical Services:
techservice.emea.genomics@thermofisher.com
Customer Services:
customerservice.emea.genomics@thermofisher.com

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