Oct 16, 2019

First strand cDNA synthesis (ThermoScientific RevertAid)

Forked from First strand cDNA synthesis (ThermoScientific RevertAid)

DOI
dx.doi.org/10.17504/protocols.io.8bmhsk6

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iGEM Wageningen 2019

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Protocol Citation: Ben Kuipers 2019. First strand cDNA synthesis (ThermoScientific RevertAid). **protocols.io https://dx.doi.org/10.17504/protocols.io.8bmhsk6**

Protocol status: Working
We use this protocol and it's working

Created: October 16, 2019

Last Modified: October 16, 2019

Protocol Integer ID: 28749

Abstract

The following protocol is optimized to generate first-strand cDNA for use in (q)PCR

Materials

MATERIALS

- 5X RT Buffer **Thermo Fisher Scientific Catalog** ##B91
- dNTP Mix 10 mM each **Thermo Fisher Scientific Catalog** ##R0191
- Water, nuclease free
- RiboLock RNase Inhibitor **Thermo Fisher Scientific Catalog** ##EO0381
- RevertAid Reverse Transcriptase (200 U/µL) **Thermo Fisher Catalog** #EP0442
- Oligo(dT)18 Primer **Thermo Fisher Catalog** #SO131

Before start

Mix and briefly centrifuge all reagents after thawing, keep on ice.

1 Add reaction components into sterile, nuclease-free tube on ice in the indicated order:

	Template RNA	100 ng (1pg - 5 µg)
	Oligo(dT)18	1 µl (100 pmol)
	Water, nuclease-free	**to 12 µl**

2 **Optional:** If the RNA template is GC-rich or is known to contain secondary structures, mix gently, centrifuge briefly and incubate at 65 °C for 5min. Chill on ice, briefly centrifuge again and place on ice
ice.

3

	5X RT Buffer	4 µl
	RiboLock RNase Inhibitor	1 µl (20 U)
	RevertAid RT (200 U/µL)	2 µl (400 U)
	10 mM dNTP Mix	1 µl
	Total volume	**20 µl**

Mix gently and centrifuge briefly.

4

	5 min	25 °C
	60 min	42 °C (For GC-rich RNA, the reaction temperature can be increased to 45 °C)
	5 min	70 °C

5 The cDNA product is now ready for downstream applications