Reverse transcription with SuperScript VI VILO

Molly A Moynihan[1]
[1]Nanyang Technological University

Dec 08, 2020

1 *Works for me* This protocol is published without a DOI.

Molly Moynihan

ABSTRACT

This protocol is from Invitrogen.
https://www.thermofisher.com/order/catalog/product/11766050#/11766050

PROTOCOL CITATION

Molly A Moynihan 2020. Reverse transcription with SuperScript VI VILO. **protocols.io** https://protocols.io/view/reverse-transcription-with-superscript-vi-vilo-bi9kkh4w

CREATED

Aug 02, 2020

LAST MODIFIED

Dec 08, 2020

PROTOCOL INTEGER ID

39948

MATERIALS TEXT

MATERIALS

SuperScript™ IV VILO™ Master Mix with ezDNase™ Enzyme **Thermo Fisher Catalog #11766500**

ABSTRACT

This protocol is from Invitrogen.
https://www.thermofisher.com/order/catalog/product/11766050#/11766050

DNase Digestion

1 Check for DNA contamination in RNA by performing PCR with universal primers (16S or 18S) (see PCR protocol). Doing the PCR first will help avoid unnecessary DNase use and wasting RNA.

OR

Proceed directly to DNase treatment without initial check.

2 **For samples with DNA contamination**

On ice, prepare 2x DNase reactions for each sample. One reaction will be used for reverse transcription (RT) and the other for the no reverse transcription control (no RT). The no RT control is used to check for DNA contamination. RT and no RT reactions should use the same amount of template RNA.

1 µl 10x ezDNase Buffer

1 µl ezDNase enzyme

up to **8 µl template RNA** (aim for 1000 - 1500ng RNA)

nuclease free water to **final volume of 10µl**

3 Gently mix and incubate samples at **37 °C** for **00:05:00** . Briefly spin down and place on ice.

4 Tip:

After RT is performed (Step 7), if DNA contamination is observed in the no RT control *and* DNase treatment was performed, try repeating the protocol with 2x DNase treatments. After the initial incubation at 37°C, add 1µl 10x ezDNase Buffer and 1µl ezDNase enzyme. Incubate again at 37°C for 5 minutes.

Reverse Transcription

5 Prepare reverse transcription reactions on ice.

Add the following to the 10µl DNase reactions above, or if no DNase treatment was performed, prepare the following in empty PCR tubes.

Each sample should have 1x RT and 1x no RT reaction. RT and no RT reactions should use the same amount of template RNA.

RT:

4 µl Superscript IV Master Mix

10 µl DNase reaction ***OR*** up to **12 µl of template RNA** (aim for 1000 - 1500ng RNA)

nuclease free water to **final volume of 20µl**

No RT controls:

4 µl Superscript IV no RT control

10 µl DNase reaction ***OR*** up to **12 µl of template RNA** (aim for 1000 - 1500ng RNA)

nuclease free water to **final volume of 20µl**

6 After closing PCR tubes, briefly spin down samples.

7 Incubate RT and no RT reactions following the settings below (7.1). This can be performed in a thermocycler.

7.1 Incubate at **25 °C** for **00:10:00** (anneal primers)

50 °C for **00:10:00** (reverse transcription)

85 °C for **00:05:00** (enzyme inactivation)

8 cDNA can be used directly in PCR or qPCR.

Store at **-20 °C** for short term storage or **-80 °C** for long term storage.