

Feb 02, 2024

SOP for RT (Reverse Transcription) Promega kit

DOI

<https://dx.doi.org/10.17504/protocols.io.8epv5x895g1b/v1>

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Protocol Citation: Malú ámez Tansey 2024. SOP for RT (Reverse Transcription) Promega kit. **protocols.io**
<https://dx.doi.org/10.17504/protocols.io.8epv5x895g1b/v1>

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Protocol status: Working

We use this protocol and it's working

Created: February 01, 2024



Last Modified: May 31, 2024

Protocol Integer ID: 94577

Keywords: ASAPCRN, promega kit sop for rt, promega kit sop, sop for rt, reverse transcription, rt, sop, kit

Funders Acknowledgements:

Aligning Science Across Parkinson's (ASAP) Collaborative Research Network

Abstract

SOP for RT (Reverse Transcription) Promega kit



Before:

- 1 Take from the kit: OligodT, RT buffer (5x), MgCl₂, dNTP mix, and H₂O to thaw- you can keep them on ice or at RT for a little while.

SOP:

- 2 Prepare per sample:

	A	B
	total RNA (1 pg-1ug)	max vol 4ul
	Water	fill up to 4ul
	OligodT	1ul
		final volume =5ul

- 3 Heat samples at 70°C for 5 min (prepare mix for step 4).
- 4 Snap freeze samples at +4°C or on ice for a minimum of 5 min.
- 5 Prepare RT mix (vol per samples, calculate for all your samples + 1):

	A	B
	RT buffer (5X)	4ul
	MgCl ₂ (25 mM)	3ul
	dNTP Mix (10 mM)	1 ul
	Rnasine*	0.5 ul
	Rtase*	1 ul
	H ₂ O	5.5ul



	A	B
		final volume= 15 ul

* add them in the last step before adding the mix to each sample. Meanwhile keep them at 20°C.

6 Brief centrifugation.

7 RT program

	A	B
	hybridization	5 min at 25°C
	elongation	60 min at 42°C
	inactivation of the enzyme	15 min at 70°C

8 Add 30 ul of H₂O to bring volume to 50 ul.

9 Once it is done, cDNA can be stored at -20°C