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# SOP for RT (Reverse Transcription) Promega kit

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

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## Abstract

SOP for RT (Reverse Transcription) Promega kit

## Before:

1 Take from the kit: OligodT, RT buffer (5x), MgCl2, dNTP mix, and $H_2O$ 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 |
| | MgCl2 (25 mM) | 3ul |
| | dNTP Mix (10 mM) | 1 ul |
| | Rnasine* | 0.5 ul |
| | Rtase* | 1 ul |
| | H2O | 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 volumne to 50 ul.

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