E.coli Poly(A) Polymerase:

Use 1-10µg of RNA for a single 20µL reaction, eluted/suspended in 15µL nuclease free water. Protocol will produce a tail length of over 100bases following these instructions. Modifying reaction time, polymerase amount and ATP concentration will modify the yield and tail length. Suspension must be free of salts.

1. Prep mastermix:

|  |  |
| --- | --- |
| **Component** | **Volume for single reaction (µL)** |
| RNA | X (use 15µL of suspension) |
| 10x E.coli Poly(A) Polymerase Reaction Buffer | 2 |
| ATP (10mM) | 2 |
| E.coli poly(A) Polymerase | 1 |
| Total volume | 20 |

2. Incubate at 37°C for 30minutes.

3. Add EDTA to a final concentration of 10mM or continuing onto the next step.

First strand synthesis:

Uses 10pg-5ug of total RNA.

1. Add components:

|  |  |
| --- | --- |
| **Component** | **Volume for single reaction (µL)** |
| Oligo(dT)12-18 primer (0.5µg/µL) | 1 |
| Poly(A) RNA- From above | 10pg-5µg of Poly-A RNA (Don’t exceed 10uL) |
| dNTPs (10mM for each dNTP) | 1 |
| Sterile Distilled water | Up to 13 |

1. Incubate at 70°C for 10minutes.
2. 4°C for 1 minute
3. Add the following:

|  |  |
| --- | --- |
| Component | Volume |
| 5x first strand buffer | 4 |
| 0.1M DTT | 1 |
| SuperScript III reverse transcriptase | 1 (200U/µL) |
| RNaseOUT RNase Inhibitor | 1 |

1. Spin down.
2. Incubate at 50°C for 30-60minutes.
3. Increase to 70°C for 15minutes to stop reaction.
4. Chill.