**cDNA synthesis – Superscript IV**

**Find a cold block**

1. Add the following for **SuperScript IV** (or equivalent) cDNA synthesis: include negative and positive control

|  |  |  |
| --- | --- | --- |
| **Component** | **Volume in 2 µL reaction** | **Master Mix for**  **12** |
| Random hexamer | 1 µL | 12 µL |
| 10 mM dNTP mix | 1 µL | 12 µL |
| Nuclease-free water | 0 µL | ---- |

2. Pipette **2uL of master mix** into strip tubes. Add **11uL RNA sample.**

3. Heat to 70°C for 7 minutes, place directly on ice (cold block) to prevent secondary structure re-forming.

4. Make second mastermix.

|  |  |  |
| --- | --- | --- |
| **Component** | **Volume in 7 µL reaction** | **Master mix for 12** |
| 5x SSIV buffer | 4 µL | 48 |
| 100 mM DTT | 1 µL | 12 |
| RNAse OUT (inhibitor) | 1 µL | 12 |
| SSIV RT (enzyme- 40U/ µL) | 1 µL | 12 |

5. Maintain the tubes on cold block and add 7 µL **master mix 2**per sample (total volume is now 20µL = 13+7) to the strip tube and mix gently, spinning briefly to bring liquid to bottom of thetube.

6**. cDNA synthesis-**Run the following cycles on athermocycler:

|  |  |
| --- | --- |
| **Temperature** | **Time** |
| 23°C | 10 minutes |
| 50°C | 45 minutes |
| 55°C | 15 minutes |
| 80°C | 10 minutes |
| 4°C | ∞ |

7. Put the samples in a cold block and Add 1µL RNAse H per sample to destroy RNA in the RNA:cDNA hybrid.

8. Incubate at **37°C for 20 minutes** (thermal cycler). Store samples at 4°C until ready for PCR.