**cDNA synthesis**

Note that this version is scaled down by half- can double all the volumes if more cDNA should be produced.

In a 0.2ml strip tube, mix the following reactions:

|  |  |  |
| --- | --- | --- |
| Reagent | Amount (ul) | Final concentration |
| Template RNA | 7.0 |  |
| Random hexamers | 1.0 | 2.5uM |

Denature the template RNA by incubating on a heat block at 65˚c for 5 minutes.

**Place immediately on ice** to prevent secondary structure formation.

Add the following reactions to each tube:

|  |  |  |
| --- | --- | --- |
| Reagent | Amount | Final concentration |
| Protoscript II Reaction Mix (2x) | 5 | 1x |
| Protoscript II Enzyme Mix (10x) | 1 | 1x |

Place on thermocycler:

|  |  |  |
| --- | --- | --- |
| Cycle number | Temperature (C) | Time |
| 1 | 25 | 5 minutes |
| 1 | 48 | 15 minutes |
| 1 | 80 | 5 minutes |

Store on ice and proceed immediately with PCR if possible.