cDNA Synthesis Protocol

James O'Callaghan

Abstract

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Protocol

Step 1.

Prepare samples by adding 4 ug of rna to rnase free water for a total of 20ul volume.

Step 2.

Prepare Master Mix 1 with 2 ul per sample of Oligo-dt primer (Thermofisher #18418012) and 2 ul per sample of dNTP mix (Thermofisher #18427088). Prepare at least 10 % excess volume to ensure sufficient quantity.

Step 3.

Add 4 ul of Master Mix 1 to each sample.

Step 4.

Incubate samples at 65 degrees Celcius for 10 minutes.

Step 5.

Place samples on ice for 5 minutes and then quickly centrifuge.

Step 6.

Prepare Master Mix 2 with 2 ul per sample of SuperScript III (Thermofisher #18080085), 2 ul per sample of Rnase Out (Thermofisher # 10777019), 8 ul per sample of 5 X first strand buffer (component of Thermofisher #18080085), and 4 ul per sample of DTT (component of Thermofisher #18080085). Prepare at least 10 % excess volume in order to ensure sufficient quantity.

Step 7.

Pipet 16 ul of Master Mix 2 per sample.

Step 8.

Incubate samples at 42 degress Celcius for 2 hours.

Step 9.

Incubate samples at 65 degrees for 5 minutes.

Step 10.

Chill samples on Ice.

Step 11.

Add 160 ul of Rnase free water to samples.

Step 12.

Store at -20 degrees Celcius.