



cDNA synthesis of Bixa orellana V.2

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Abstract

The SuperScript™ III First-Strand Synthesis System for RT-PCR is optimized to synthesize first-strand cDNA from purified poly(A)+ or total RNA.

Materials

MATERIALS

⊗ SuperScript™ III Reverse Transcriptase **Invitrogen - Thermo Fisher Catalog #Cat. No. 18080093**

⊗ RNase OUT™ Recombinant RNase Inhibitor **Invitrogen - Thermo Fisher Catalog #Cat. No. 10777-019**

NanoDrop® ND-1000

SuperScript™ III Reverse Transcriptase (Invitrogen, Cat. No. 18080-093)

random hexamers

RNA inhibitors (RNase OUT™ Recombinant RNase Inhibitor, Cat. No. 10777-019)



- 1 The synthesis of cDNA was carried out from 100 ng of total RNA using the enzyme SuperScript™ III Reverse Transcriptase (Invitrogen, Cat. No. 18080-093)
In summary, in a $.5\text{ mL}$ nuclease-free Eppendorf tube, $1\text{ }\mu\text{L}$ of dNTPs mixture (containing dATP, dGTP, dCTP each at a concentration of 10mM of each nucleotide), of total RNA, random hexamers as initiators and sterile doubly distilled water free of nucleases was mixed in a final volume of $0.5\text{ }\mu\text{L}$. This reaction mixture was heated to $65\text{ }^{\circ}\text{C}$ for $00:05:00$ and incubated on ice for $00:01:00$, before adding $4\text{ }\mu\text{L}$ First-Strand 5X buffer, $1\text{ }\mu\text{L}$ DTT [1M] 0.1 Mass Percent, $1\text{ }\mu\text{L}$ Invitrogen RNA inhibitors (RNase OUT™ Recombinant RNase Inhibitor, Cat. No. 10777-019, 40 U/ μL) and $1\text{ }\mu\text{L}$ reverse transcriptase (SuperScript™ III RT 200 U/ μL) for a total volume of $20\text{ }\mu\text{L}$. Subsequently, the reaction was incubated at room temperature for $00:05:00$ and at $55\text{ }^{\circ}\text{C}$ for $01:00:00$, and finally the enzyme was inactivated by heating the reaction to $70\text{ }^{\circ}\text{C}$ for $00:15:00$. The reactions were stored at $-20\text{ }^{\circ}\text{C}$.