# [General protocol] Reverse Transcription cDNA Synthesis

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General protocol for RT cDNA synthesis from RNA extract
Version: 1.0 (10.05.2023)

**Media and Reagents:**
RNA samples
RNase/DNase-free water
All other media and reagents are found in the SuperScript Kit

**Materials and Equipment:**
SuperScript™ III First-Strand Synthesis SuperMix
0.2-mL, nuclease-free, thin-walled PCR tubes
Thermal cycler
Ice
Cold PCR rack
Microcentrifuge
Vortex mixer

**1. Introduction and Purpose**
This protocol describes the procedure for reverse transcribing RNA extracts into cDNA.

**2. Protocol for cDNA Synthesis**
Before starting, take an ice box and a cold PCR rack. Preheat the thermal cycler to 65°C.
Keep all components, reaction mixes, and samples on ice or a cold PCR rack throughout the protocol.
The kit can convert 0.1 pg to 5 µg of total RNA, the amount should be maximum 5 µg in tube.

1. Thaw, vortex, and briefly centrifuge all components that will be used.
2. Pipette the following in a 0.2 mL PCR tube for each separate RNA extract:
   - 0.5 uL Random hexamer mix
   - 0.5 uL Annealing buffer
   - 0.5 uL Nuclease-free water
   - 2.5 uL RNA sample (up to 5 µg RNA)
3. Incubate tube(s) in a thermal cycler at 65°C for 5 minutes, then immediately place on ice for at least 1 minute. Collect the contents of the tube(s) by brief centrifugation.
4. Pipette the following on each PCR tube:
   - 5 uL Reaction mix
   - 1 uL Enzyme mix
5. Vortex and briefly centrifuge the PCR tubes.
6. Place tubes in the thermal cycler and start the cDNA synthesis template protocol created by Ama. The program is 5 mins at 25°C, 50 mins at 50°C, 5 mins at 80°C and cool down to 0°C.
7. Store the tubes at –20°C, or proceed directly to PCR.