

# Quick Reference Card

## High Capacity RNA-to-cDNA Kit

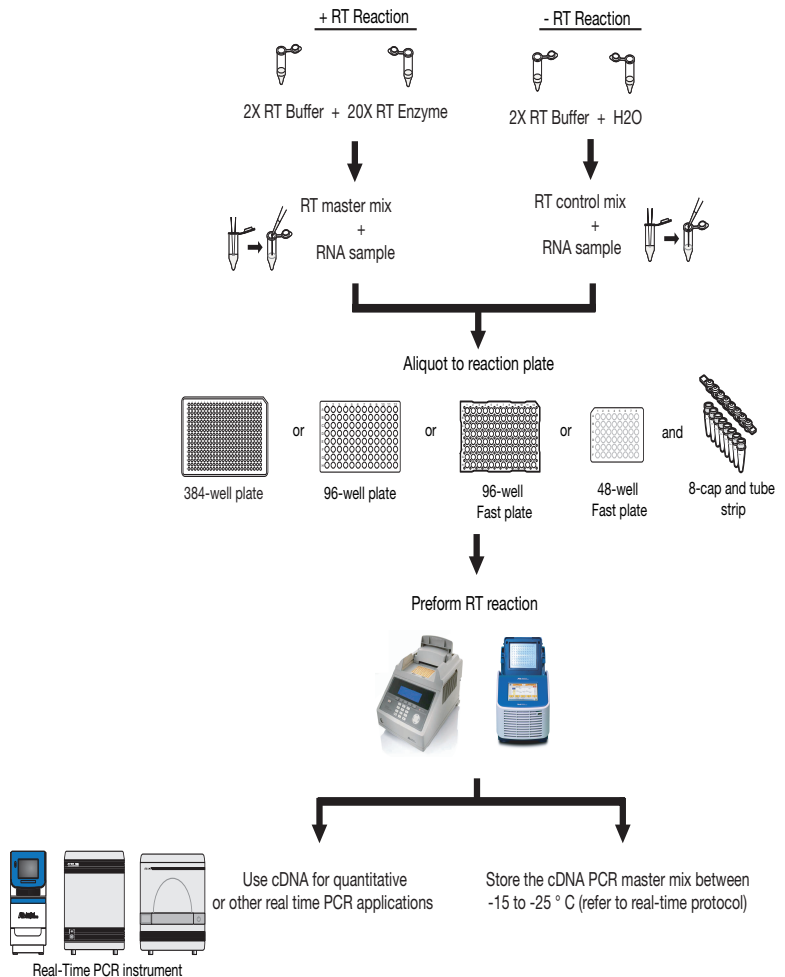
For safety and biohazard guidelines, refer to the “Safety” section in the *High Capacity RNA-to-cDNA Kit Protocol*. For all chemicals in **bold red type**, read the MSDS and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves.

### Overview


To synthesize single-stranded cDNA from total RNA using the High Capacity RNA-to-cDNA Kit:

For optimal performance of the High Capacity RNA-to-cDNA Kit, Applied Biosystems recommends using RNA that is:

- Free of inhibitors of reverse transcription and PCR
- Dissolved in TE Buffer or PCR-compatible buffer
- Free of RNase activity.



## Procedure

STEP	ACTION																						
1	Input Amount of Total RNA	Use up to 2 µg of total RNA per 20 µL reaction.																					
2	Preparing the RT reaction	<p>Prepare the RT reaction mix using the kit components before preparing the reaction plate.</p> <p>To prepare the RT reaction mix (per 20 µL reaction):</p> <ol style="list-style-type: none"> <li>Allow the kit components to thaw on ice.</li> <li>Referring to the following table, calculate the volume of components needed to prepare the required number of reactions.</li> </ol> <table border="1" data-bbox="549 539 1224 852"> <thead> <tr> <th data-bbox="549 539 838 591">Component</th> <th colspan="2" data-bbox="838 539 1224 591">Volume/Reaction (µL)</th> </tr> <tr> <td data-bbox="549 591 838 635"></td> <td data-bbox="838 591 1030 635">+RT</td> <td data-bbox="1030 591 1224 635">-RT</td> </tr> </thead> <tbody> <tr> <td data-bbox="549 635 838 678"><b>2X RT Buffer</b></td> <td data-bbox="838 635 1030 678">10.0</td> <td data-bbox="1030 635 1224 678">10.0</td> </tr> <tr> <td data-bbox="549 678 838 722">20X RT Enzyme Mix</td> <td data-bbox="838 678 1030 722">1.0</td> <td data-bbox="1030 678 1224 722">—</td> </tr> <tr> <td data-bbox="549 722 838 765">Nuclease-free H<sub>2</sub>O</td> <td data-bbox="838 722 1030 765">Q.S. † to 20 µL</td> <td data-bbox="1030 722 1224 765">Q.S. to 20 µL</td> </tr> <tr> <td data-bbox="549 765 838 808">Sample</td> <td data-bbox="838 765 1030 808">up to 9 µL</td> <td data-bbox="1030 765 1224 808">up to 9 µL</td> </tr> <tr> <td data-bbox="549 808 838 852">Total per Reaction</td> <td data-bbox="838 808 1030 852">20.0</td> <td data-bbox="1030 808 1224 852">20.0</td> </tr> </tbody> </table> <p data-bbox="549 861 733 887">† Quantity Sufficient</p> <p data-bbox="518 927 1251 1008"><b>Note:</b> Prepare the RT reaction on ice. Include additional reactions in the calculations to provide excess volume for the loss that occurs during reagent transfers.</p> <p data-bbox="518 1013 1251 1156"> <b>WARNING CHEMICAL HAZARD.</b> <b>2X RT Buffer</b> may cause eye, skin, and respiratory tract irritation. Read the MSDS, and follow the handling instructions. Wear appropriate eyewear, clothing, and gloves.</p>	Component	Volume/Reaction (µL)			+RT	-RT	<b>2X RT Buffer</b>	10.0	10.0	20X RT Enzyme Mix	1.0	—	Nuclease-free H <sub>2</sub> O	Q.S. † to 20 µL	Q.S. to 20 µL	Sample	up to 9 µL	up to 9 µL	Total per Reaction	20.0	20.0
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3	Preparing the cDNA Reverse Transcription Reactions	<p>To prepare the cDNA RT reactions:</p> <ol style="list-style-type: none"> <li>Aliquot 20 µL of RT reaction mix into each well, or tube.</li> <li>seal the plates or tubes.</li> <li>Briefly centrifuge the plate or tubes to spin down the contents and to eliminate any air bubbles.</li> </ol> <p>Place the plate or tubes on ice until you are ready to load the thermal cycler or Applied Biosystems Real-Time PCR system.</p>																					

STEP	ACTION														
<p><b>4</b></p>	<p>Performing Reverse Transcription</p>	<p>To perform reverse transcription:</p> <p>a. Using one of the required thermal cyclers listed in “Materials and Equipment” in <i>High Capacity RNA-to-cDNA Kit Protocol</i> (part number 4387951), program the thermal cycler conditions:</p> <p><b>IMPORTANT!</b> These conditions are optimized for use with the High Capacity RNA-to-cDNA Kit.</p> <table border="1" data-bbox="521 388 1241 519"> <thead> <tr> <th></th> <th>Step 1</th> <th>Step 2</th> <th>Step 3</th> </tr> </thead> <tbody> <tr> <td>Temperature (°C)</td> <td>37</td> <td>95</td> <td>4</td> </tr> <tr> <td>Time</td> <td>60 min</td> <td>5 min</td> <td>∞</td> </tr> </tbody> </table> <p>b. Set the reaction volume to <b>20 µL</b>.</p> <p>c. Load the reactions into the thermal cycler or Applied Biosystems Real-Time PCR system.</p> <p>d. Start the reverse transcription run.</p>			Step 1	Step 2	Step 3	Temperature (°C)	37	95	4	Time	60 min	5 min	∞
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<p><b>5</b></p>	<p>Storing cDNA Reverse Transcription Reactions</p>	<p>You can store cDNA RT plates or tubes prepared using the High Capacity RNA-to-cDNA Kit for short-term or long-term storage.</p> <table border="1" data-bbox="516 772 1260 914"> <thead> <tr> <th>Storage Duration</th> <th>Storage Temperature (°C)</th> </tr> </thead> <tbody> <tr> <td>Short-term (up to 24 hours before use)</td> <td>2 to 8</td> </tr> <tr> <td>Long-term</td> <td>-15 to -25</td> </tr> </tbody> </table> <p>If required, briefly centrifuge the archive plates or tubes before storing to spin down the contents and to eliminate any air bubbles.</p>		Storage Duration	Storage Temperature (°C)	Short-term (up to 24 hours before use)	2 to 8	Long-term	-15 to -25						
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