

 **Package contents**      **Catalog No.** A45003      **Size** 32 reaction       **Kit contents**

 **Storage conditions**      Store all contents at  $-30^{\circ}\text{C}$  to  $-10^{\circ}\text{C}$  (non-frost-free)


 **Required materials**

- Template: RNA


 **Timing**

- Preparation time: 5 minutes
- Total incubation time: 25 minutes

The Ion Torrent™ NGS RT Kit is the first cDNA synthesis kit developed specifically for next-generation sequencing (NGS) applications with specific validated assays. The kit contains 2 components:

 **Product description**

- The 10X RT Enzyme Mix includes SuperScript™ IV Reverse Transcriptase, a proprietary RNase inhibitor, helper proteins, and stabilizer proteins.
- The 5X Reaction Buffer contains dNTPs, random hexamers, and  $\text{MgCl}_2$  in a formulation optimized for NGS library preparation.

 **Online resources**      Visit our **product page** for additional information, protocols, and Certificates of Analysis (CoA).  
For support, visit [thermofisher.com/support](http://thermofisher.com/support).

## Guidelines for RNA preparation

This kit can be used to prepare cDNA for a variety of Ion Torrent™ NGS assays and is compatible with many different sample types. Consult the assay-specific user guide for recommended RNA extraction kits and sample preparation guidelines.

## Guidelines for reverse transcription

Use up to 2.5  $\mu\text{g}$  of total RNA as starting material in a 10- $\mu\text{L}$  reaction.

Note: Input amounts and reaction volumes vary with each assay and sample type. Consult assay-specific user guides for recommendations.

Note: To avoid PCR inhibition, RT reaction volume should be equal to or less than 50% of the PCR reaction volume. For example, use a 10- $\mu\text{L}$  RT as input for a 20- $\mu\text{L}$  PCR reaction.

## Safety

Read the Safety Data Sheets (SDSs) and follow the handling instructions. Wear appropriate protective eyewear, clothing, and gloves. Safety Data Sheets (SDSs) are available from [thermofisher.com/support](http://thermofisher.com/support).

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## Reverse transcription protocol for Ion Torrent™ NGS Reverse Transcription Kit

Step	Action	Procedure details												
1	<b>Add components to well and prepare master mix (on ice)</b>	<p>For each RT reaction, add the following components into a single well of a 96-well PCR plate on ice or in a pre-chilled 4°C cold block. Prepare a master mix without sample RNA for multiple reactions.</p> <p>Note: We recommend that you reverse-transcribe a positive control RNA sample and a no-template control to help answer questions concerning overall reverse transcription performance, PCR inhibitors present in the sample, or contamination.</p> <table border="1"> <thead> <tr> <th>Component</th> <th>Volume</th> </tr> </thead> <tbody> <tr> <td>Ion Torrent™ NGS 5X Reaction Buffer</td> <td>2 µL</td> </tr> <tr> <td>Ion Torrent™ NGS 10X RT Enzyme Mix</td> <td>1 µL</td> </tr> <tr> <td>Total RNA (100 pg to 2.5 µg)<sup>[1,2]</sup></td> <td>≤7 µL</td> </tr> <tr> <td>Nuclease-free Water</td> <td>to 10 µL</td> </tr> <tr> <td><b>Total volume per well</b></td> <td><b>10 µL</b></td> </tr> </tbody> </table> <p><sup>[1]</sup> Substitute an equal volume of nuclease-free water or Low TE to prepare a no-template control (NTC).  <sup>[2]</sup> Consult the assay-specific user guide for recommendations on optimal input amount for your assay and sample type.</p>	Component	Volume	Ion Torrent™ NGS 5X Reaction Buffer	2 µL	Ion Torrent™ NGS 10X RT Enzyme Mix	1 µL	Total RNA (100 pg to 2.5 µg) <sup>[1,2]</sup>	≤7 µL	Nuclease-free Water	to 10 µL	<b>Total volume per well</b>	<b>10 µL</b>
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2	<b>Seal the plate and mix</b>	Seal the plate with MicroAmp™ Adhesive Film, vortex thoroughly, then briefly centrifuge to collect droplets. Alternatively, mix by pipetting at least half the total volume up and down at least 5 times before sealing the plate.												
3	<b>Synthesize cDNA</b>	<p>Place a MicroAmp™ Compression Pad on the plate, load the plate in the thermal cycler, then run the following program to synthesize cDNA.</p> <table border="1"> <thead> <tr> <th>Temperature</th> <th>Time</th> </tr> </thead> <tbody> <tr> <td>25°C</td> <td>10 minutes</td> </tr> <tr> <td>50°C</td> <td>10 minutes</td> </tr> <tr> <td>85°C</td> <td>5 minutes</td> </tr> <tr> <td>10°C</td> <td>Hold</td> </tr> </tbody> </table> <p>Note: Samples can be stored at 10°C for up to 16 hours in the thermal cycler..</p>	Temperature	Time	25°C	10 minutes	50°C	10 minutes	85°C	5 minutes	10°C	Hold		
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4	<b>Centrifuge and collect droplets</b>	<p>Briefly centrifuge the plate to collect any droplets at the bottom of the wells.</p> <p>Note: For long-term storage, transfer the cDNA to an RNase-free microcentrifuge tube and store at –20°C.</p>												