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SuperScript® One-Cycle cDNA Kit for Use with Affymetrix® One-Cycle Assays

Cat. no. A10752-030 **Size: 30 reactions** **Store at –20°C**

Kit Components

The following components are provided with the kit. Store all reagents at –20°C.

Component	Amount
T7-Oligo(dT), 50 µM	60 µl
5X 1st-Strand Reaction Mix	120 µl
DTT, 0.1 M	60 µl
dNTP Mix, 10 mM (10 mM each dATP, dCTP, dGTP, dTTP)	120 µl
SuperScript® II RT (200 U/µl)	60 µl
5X 2nd-Strand Reaction Mix	900 µl
E. coli DNA Ligase (10 U/µl)	30 µl
E. coli DNA Polymerase I (10 U/µl)	120 µl
RNase H (2 U/µl)	30 µl
T4 DNA Polymerase (5 U/µl)	60 µl
RNase-free water	3.1 ml
EDTA, 0.5 M	300 µl

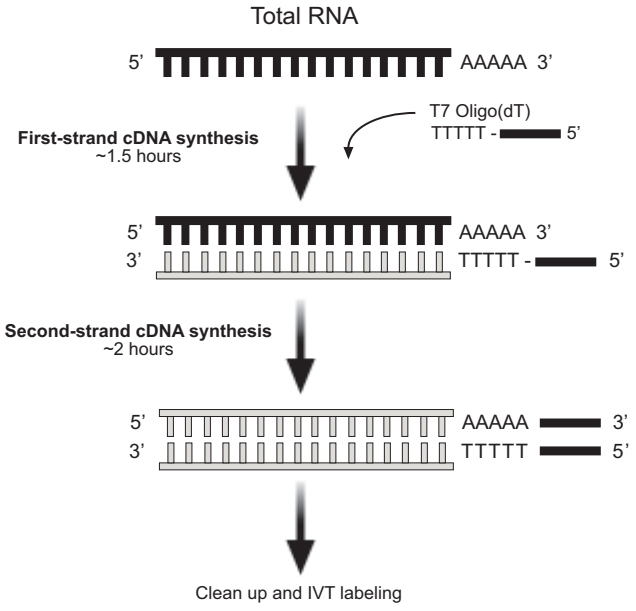
Description

The SuperScript® One-Cycle cDNA Kit contains all of the reagents necessary to make double-stranded cDNA from purified total RNA or mRNA for use with the GeneChip® One-Cycle Target Labeling System from Affymetrix. This kit is designed to fit into the standard one-cycle labeling protocol for GeneChip® arrays from Affymetrix, as described in the GeneChip® Expression Analysis Technical Manual.

It is important to use high-quality RNA isolated from tissue or cells for your cDNA preparation. High-quality RNA can be obtained by isolating total RNA with the PureLink™ Micro-to-Midi Total RNA Purification System or TRIzol® Reagent. See page 4 for ordering information.

Workflow Overview

Following isolation of total RNA or mRNA from tissue or cells, use this kit to generate double-stranded cDNA as shown in the workflow below. Then proceed directly to purification using the GeneChip® Sample Cleanup Module from Affymetrix.



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Before Starting

- Briefly centrifuge all reagents in the kit before use.
- For best results, use a thermocycler for all incubation steps.
- RNA can be quantified using UV absorbance at 260 nm or a quantitation kit such as the Quant-iT™ RNA Assay Kit. RNA quality can be assessed by agarose gel electrophoresis or on an Agilent 2100 Bioanalyzer.

First-Strand Synthesis

The 20-µl reaction described in this protocol is designed to convert 1–15 µg of total RNA or 0.2–2 µg of mRNA into first-strand cDNA. The amount of SuperScript® II RT added to the reaction will depend on the amount of starting RNA.

- **Total RNA:** We recommend using 200 units of SuperScript® II RT for <8 µg total RNA or 400 units for 8–15 µg total RNA.
- **mRNA:** We recommend using 200 units of SuperScript® II RT per µg of mRNA.
- **Poly-A RNA controls:** The GeneChip® Eukaryotic Poly-A RNA Control Kit provides poly-A RNA controls specifically designed for the Affymetrix® GeneChip® One-Cycle Target Labeling system. If you are using these controls, dilute them as described in the GeneChip® Expression Analysis Technical Manual, then add 2 µl of the diluted controls per reaction into the first-strand synthesis reaction as shown in Step 1 below.

1. For each reaction, add T7 oligo(dT) primer, RNA, RNase-free water, and optional controls according to the table below to a RNase-free 1.5-ml microcentrifuge tube:

Component	Volume if using <8 µg Total RNA *or* <1 µg mRNA	Volume if using 8–15 µg Total RNA *or* 1–2 µg mRNA
Sample RNA (µl)	variable	variable
Optional: Diluted GeneChip® Eukaryotic Poly-A RNA Controls	2 µl	2 µl
T7-oligo(dT), 50 µM	2 µl	2 µl
RNase-free water	to 12 µl	to 11 µl

2. Flick the tube gently to mix, then centrifuge briefly to collect the contents. Heat the mixture to 70°C for 10 minutes, and then chill on ice.
3. In a separate tube, prepare a master mix of the following reagents. Multiply the number of reactions by the volume per reaction listed in the table below; be sure to prepare extra master mix to allow for pipetting variations.

Component	Volume per reaction
5X 1st-Strand Reaction Mix	4 µl
DTT, 0.1 M	2 µl
dNTP Mix, 10 mM	1 µl
Total volume	7 µl

4. Mix the master mix gently by flicking the tube and centrifuge briefly to collect the contents. Add 7 µl of the first-strand master mix to each reaction tube.
5. Vortex gently and then centrifuge briefly to collect the tube contents. Immediately incubate each tube at 42°C for 2 minutes.
6. Add SuperScript® II RT to each tube as shown in the following table, to bring the total volume to 20 µl:

Component	Volume if using <8 µg Total RNA *or* <1 µg mRNA	Volume if using 8–15 µg Total RNA *or* 1–2 µg mRNA
SuperScript® II RT	1 µl	2 µl

7. Mix gently, and incubate at 42°C for 1 hour.
8. Place the tube on ice to terminate the reaction, and proceed to **Second-Strand Synthesis**.

Second-Strand Synthesis

1. In a separate tube, prepare a master mix of the following reagents. Multiply the number of reactions by the volume per reaction listed in the table below; be sure to prepare extra master mix to allow for pipetting variations.

Component	Volume per reaction
RNase-free water	91 µl
5X 2nd-Strand Reaction Mix	30 µl
dNTP Mix, 10 mM	3 µl
E. coli DNA Ligase (10 U/µl)	1 µl
E. coli DNA Polymerase I (10 U/µl)	4 µl
RNase H (2 U/µl)	1 µl
Final volume	130 µl

2. Mix the master mix gently by flicking the tube and centrifuge briefly to collect the contents. On ice, add 130 µl of the master mix to each 20-µl first-strand reaction tube.
3. Vortex gently to mix, and incubate for 2 hours at 16°C. Do not allow the temperature to rise above 16°C.
3. Add 2 µl (10 units) of T4 DNA Polymerase, and continue to incubate at 16°C for 5 minutes.
4. Place the tube on ice, and add 10 µl of EDTA, 0.5 M, to stop the reaction.
5. Proceed to cleanup of the double-stranded cDNA product using the GeneChip® Sample Cleanup Module from Affymetrix, followed by *in vitro* transcription and target labeling as described in the GeneChip® Expression Analysis Technical Manual. If you are not immediately proceeding to cleanup, store the double-stranded cDNA at –20°C.

Product Qualification

The Certificate of Analysis (CofA) provides detailed quality control information for this product. You can search for the Certificate of Analysis on our product support page at www.invitrogen.com/support. Enter the product lot number in the search field and select the **Certificates of Analysis** search. Note that the lot number is printed on each box.

Additional Products

Related products are available separately from Invitrogen. Ordering information is provided below. For more information, visit our website at **www.invitrogen.com** or contact Technical Support.

Product	Amount	Catalog no.
Quant-iT™ RNA Assay Kit	1 kit	Q-33140
PureLink™ Micro-to-Midi™ Total RNA Purification System	50 rxns	12183-018
TRIzol® Reagent	100 ml 200 ml	15596-026 15596-018

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