applied biosystems PRODUCT INFORMATION SHEET

GeneChip™ T7-Oligo(dT) Promoter Primer Kit

Catalog Number 900375

Pub. No. 701182 **Rev.** 3

WARNING! 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**.

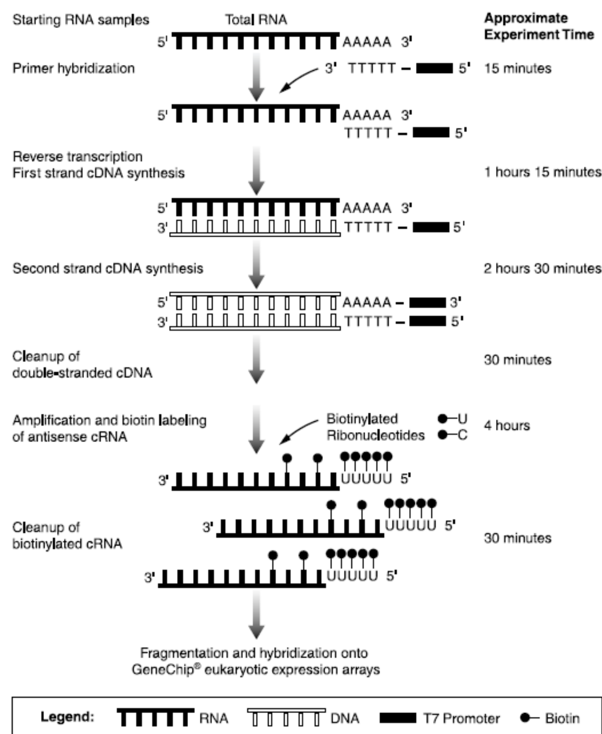
Product use

The Applied Biosystems™ GeneChip™ T7-Oligo(dT) Promoter Primer Kit provides reagents essential for the primer hybridization step in the first strand cDNA synthesis reaction of eukaryotic target preparation.

Introduction

In situ synthesized oligonucleotide probes on GeneChip™ eukaryotic expression arrays are designed in the sense orientation of corresponding transcripts. In GeneChip™ eukaryotic expression analysis, sample amplification and labeling is used to produce biotinylated antisense targets for hybridization to probes on the arrays. Instructions for the primer hybridization step are described below. The remaining procedure for target preparation is in the *GeneChip™ Expression Analysis Technical Manual* (Technical Manual), available on our website and will not be duplicated here.

The complete procedure involves hybridizing a chimeric T7-oligo(dT) primer to the sample mRNA in the reverse transcription reaction for first strand cDNA synthesis, followed by second strand cDNA synthesis. The purified double-stranded cDNA containing a T7 promoter at its 5′ end can now serve as a template in the *in vitro* transcription reaction that produces many copies of complementary RNA (cRNA) that has incorporated biotinylated nucleotides. The biotinylated cRNA targets are then fragmented and hybridized to GeneChip™ expression arrays.



The GeneChip™ T7-Oligo(dT) Promoter Primer Kit provides the essential reagents that are used in the primer hybridization step of the first strand cDNA synthesis reaction. This kit has modified the stock concentration of the T7-oligo(dT) primer to 50 µM from 100 µM as previously recommended. Use the following procedure for the primer hybridization step, then follow the protocols that are described in the Technical Manual for the subsequent cDNA synthesis and cRNA amplification.

Kit components and storage

Unless otherwise indicated, all materials are available through **thermofisher.com**.

MLS: Fisher Scientific (**fisherscientific.com**) or other major laboratory supplier.

Cat. No.	Size	Components
900375	150 reactions	50 µM T7-oligo(dT) primer, HPLC purified 300 µL × one vial (sequence: 5′ – GGCCAGTGAATTGTAATACG ACTCACTATAGGGAGGCGG –$(dT)_{24}$ –3′)
		RNase-free water 825 µL × 2 vials

IMPORTANT! The primer should be stored at –20°C. Performance has been shown to be unaffected for up to 25 freeze-thaw cycles.

For Research Use Only. Not for use in diagnostic procedures.

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Materials not provided with the kit

See the Technical Manual for a complete list of reagents that are needed to complete cDNA synthesis, cleanup, and cRNA amplification and labeling.

Before starting

The quality of the RNA samples is essential to the overall success of the analysis. We recommend routine assessment of RNA quality and integrity for all samples.

Either eukaryotic total RNA or poly-A RNA can be used as starting material for GeneChip™ target labeling. A minimum amount of 5 µg of total RNA or 0.2 µg of poly-A RNA is recommended.

Follow routine laboratory procedures for handling RNA samples and components of this kit to prevent RNase contamination.

Primer hybridization procedure

1. Remove the T7-oligo(dT) primer from the freezer. Ensure that the primer is thawed before proceeding.
2. Lightly vortex the vial of primer for complete mixing. Briefly spin down the reagent in a microcentrifuge.
3. In a microfuge tube, combine sample RNA with 2 µL of 50 µM T7-oligo(dT) primer.
4. Calculate the amount of RNase-free water that is needed to make up a final volume of 20 µL for the entire first strand cDNA synthesis reaction. See the Technical Manual for the volume calculation. Add the appropriate amount of RNase-free water to the reaction.
5. Heat the reaction mix at 70°C for 10 minutes to denature the sample RNA/promoter primer mix.
6. Quickly spin the microfuge tube and immediately cool on ice.
7. The RNA/primer complex is now ready for cDNA synthesis. See the Technical Manual for complete GeneChip™ target amplification instructions.

Customer and technical support

Visit **thermofisher.com/support** for the latest in services and support, including:

- Worldwide contact telephone numbers
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  - Product FAQs
  - Software, patches, and updates
  - Training for many applications and instruments
- Order and web support
- Product documentation, including:
  - User guides, manuals, and protocols
  - Certificates of Analysis
  - Safety Data Sheets (SDSs; also known as MSDSs)

  Note: For SDSs for reagents and chemicals from other manufacturers, contact the manufacturer.

Limited product warranty

Life Technologies Corporation and/or its affiliate(s) warrant their products as set forth in the Life Technologies' General Terms and Conditions of Sale found on Life Technologies' website at **www.thermofisher.com/us/en/home/global/terms-and-conditions.html**. If you have any questions, please contact Life Technologies at **www.thermofisher.com/support**.

Thermo Fisher Scientifc Baltics UAB | V.A. Graiciuno 8, LT-02241 | Vilnius, Lithuania

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Revision	Date	Description
3	06 September 2017	Update document to current template.
2	07 November 2002	Baseline for revision history.

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