



GeneChip® Expression 3'-Amplification Reagents Two-Cycle cDNA Synthesis Kit

P/N 900432 (30 eukaryotic reactions)

Manufactured by Invitrogen for Affymetrix.

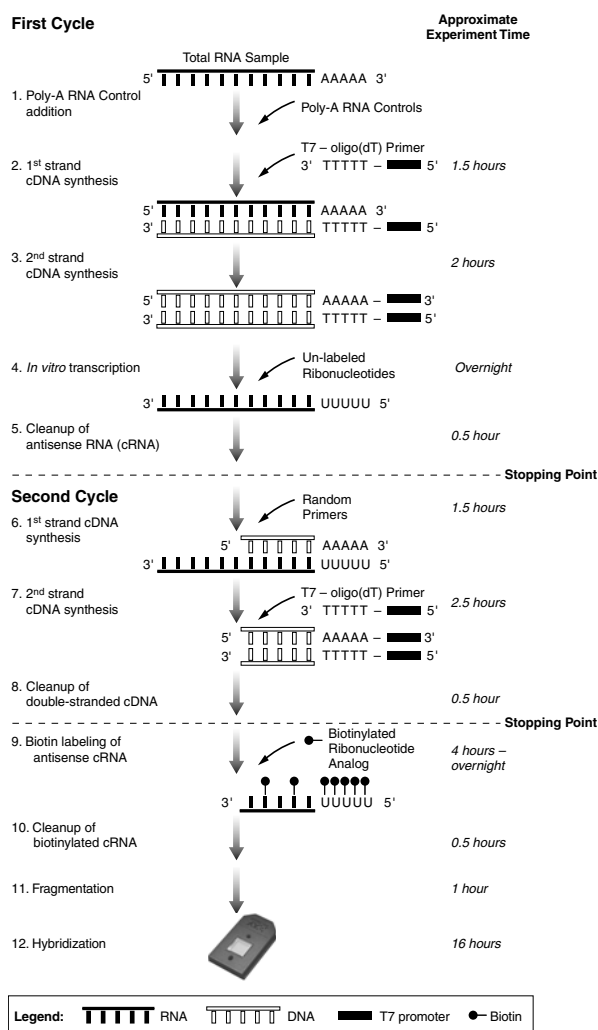
INTRODUCTION

The Two-Cycle cDNA Synthesis Kit is optimized specifically for GeneChip® eukaryotic target preparation. It contains sufficient reagents to carry out two cycles of cDNA synthesis (not including the intermediate *in vitro* transcription (IVT) amplification reactions) from 10 to 100 ng of total RNA. Sufficient quantity of double-stranded cDNA can be obtained and purified, and is used as a template in the second IVT reaction to generate biotin-labeled complementary RNA (cRNA). The biotinylated cRNA targets are then cleaned up, fragmented, and hybridized to GeneChip brand expression arrays.

Detailed procedure for this assay is described in the *GeneChip® Expression Analysis Technical Manual*; the latest update is available at www.affymetrix.com.

For starting material higher than 1 µg of total RNA, the GeneChip One-Cycle cDNA Synthesis Kit (P/N 900431) can be used.

Two-Cycle Target Labeling Assay



OTHER NECESSARY REAGENTS

NOT PROVIDED WITH THE KIT _____

The following GeneChip brand reagents are necessary for performing the Two-Cycle Target Labeling Assay, some of which need to be ordered separately from the Two-Cycle cDNA Synthesis Kit.

Kit Name	P/N	Steps the kit is used for in the assay	Size
Eukaryotic Poly-A RNA Control Kit*	900433	Step 1	~ 100 rxns
Two-Cycle cDNA Synthesis Kit	900432	Steps 2, 3, 6, and 7	30 rxns
Sample Cleanup Module*	900371	Steps 5, 8, 10, and 11	15 rxns
GeneChip® IVT Labeling Kit*	900449	Step 9	30 rxns
Hybridization Controls*	900454 and 900457	Step 12	30 rxns and 150 rxns

* Reagents that need to be ordered separately

CONTENTS

Volume	Component
120 µL	T7-Oligo(dT) Primer, 50 µM
180 µL	5X 1st Strand Rxn Mix
90 µL	DTT, 0.1M
200 µL	dNTP, 10 mM
45 µL	RNase Inhibitor
60 µL	SuperScript™ II
900 µL	5X 2nd Strand Rxn Mix
200 µL	<i>E. coli</i> DNA Polymerase I
60 µL	MgCl ₂ , 1M
55 µL	RNase H
60 µL	Random Primers
60 µL	T4 DNA Polymerase
9.5 mL	RNase-free Water

STORAGE

Kit should be kept at -20°C in a non-frost-free freezer. Performance of the kit has been shown to be unaffected for up to twenty freeze-thaw cycles.

FUNCTIONAL TESTING

GeneChip Array Functional Testing

Each lot is functionally tested in the GeneChip expression assay following the recommended protocol.

SAFETY INFORMATION

An MSDS is available from Affymetrix. The chemical, physical, and toxicological properties of these components have not been thoroughly investigated.

CONTACT INFORMATION _____

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TRADEMARKS _____

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SuperScript™ is a trademark of Invitrogen Corporation.

PATENTS _____

USE OF THIS KIT IN ACCORDANCE WITH THESE INSTRUCTIONS IN
PREPARING TARGETS FOR GENECHIP® PROBE ARRAYS MAY BE COVERED BY
U.S. PATENT NUMBERS 5,545,522; 5,716,785; 5,891,636 AND 6,291,170.
THOSE WHO DO NOT USE THIS KIT TO PREPARE TARGETS FOR GENECHIP®
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