

## GeneChip® HT IVT Labeling Kit

For use with the GeneChip®  
Array Station

P/N 900688  
(96 eukaryotic reactions)  
In vitro transcription with  
MEGAscript® reagents



### INTRODUCTION

The GeneChip® HT IVT Labeling Kit is optimized specifically for producing amplified and biotinylated targets to hybridize to eukaryotic GeneChip® brand arrays for expression analysis when using the GeneChip® Array Station. Each kit contains sufficient reagents for completing 96 *in vitro* transcription reactions when following the detailed procedures described in the *GeneChip® Expression Analysis Technical Manual (For Cartridge Arrays Using the GeneChip Array Station)*. The latest update of Technical Manual (P/N 702064) is available at [www.affymetrix.com](http://www.affymetrix.com).

The template DNA for this IVT reaction is typically double-stranded cDNA containing the T7 promoter sequence. In GeneChip target labeling experiments, the T7 promoter sequence is incorporated into the cDNA template by using a T7-Oligo(dT) Promoter Primer in the initial reverse transcription reaction.

Using this HT IVT Labeling Kit with MEGAscript® reagents (IVT Labeling Enzyme Mix and T7 RNA Polymerase), biotinylated cRNA targets are generated in the presence of an optimized mixture of the four natural ribonucleotides and one biotin-conjugated nucleotide analog (IVT Labeling NTP Mix). The chemical structure of the analog is illustrated in Figure 1.

The synthetic analog is efficiently incorporated into the cRNA target during the *in vitro* transcription labeling reaction mediated by T7 RNA polymerase as a pseudouridine reagent. The biotinylated cRNA targets are then purified, fragmented, and hybridized to GeneChip expression arrays.

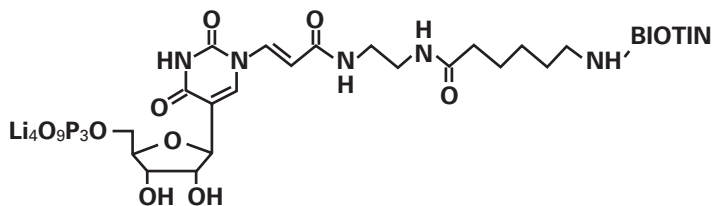


Figure 1. Structure of the biotin-conjugated nucleotide analog

### KIT COMPONENTS

#### GeneChip® HT IVT Labeling Kit

Volume	Component	
675 µL	10X IVT Labeling Buffer	1 tube
675 µL	IVT Labeling Enzyme Mix	1 tube
1,010 µL	IVT Labeling NTP Mix	2 tubes
10 µL	3'-Labeling Control (0.5 µg/µL)	1 tube
115 µL	T7 RNA Polymerase	1 tube
855 µL	5X Fragmentation Buffer	1 tube

#### OTHER NECESSARY REAGENTS NOT PROVIDED WITH THE KIT

Nuclease-free water is also necessary for performing the HT IVT Labeling reaction. We recommend nuclease-free water from Ambion, P/N 9932.

#### ADDITIONAL AFFYMETRIX® REAGENTS

The following reagents are necessary for performing the GeneChip expression assay.

Affymetrix Reagents	P/N	Steps	Size
GeneChip® Eukaryotic Poly-A RNA Control Kit	900433	Step 1	~ 100 rxns
GeneChip® HT One-Cycle cDNA Synthesis Kit	900687	Step 2, 3	96 rxns
GeneChip® Eukaryotic Hybridization Control Kit	900454 or 900457	Step 8	30 rxns, 150 rxns

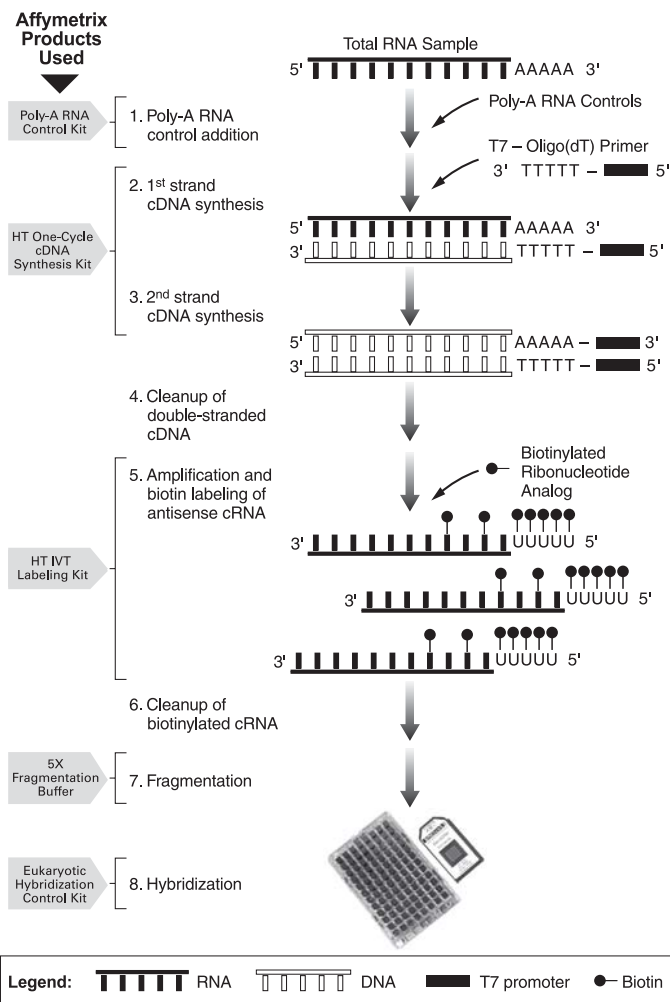


Figure 2. Schematic illustration of GeneChip® target labeling assays for expression analysis using the GeneChip® Array Station

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## STORAGE

The kit should be kept at -20°C in a non-frost-free freezer.

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**IMPORTANT** ! **Store all reagents in a -20°C freezer that is not self-defrosting. Prior to use, centrifuge all reagents briefly to ensure that the solution is collected at the bottom of the tube.**

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## FUNCTIONAL TESTING AND QUALITY CONTROL

Each lot is functionally tested with the GeneChip expression assay.

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## SAFETY INFORMATION

Material Safety Data Sheet(s) will be sent only on the first shipment, unless a substantial revision occurs. If the product is a kit or is supplied with more than one material, please refer to the MSDS for each component for hazard information.

For detailed safety information regarding the operation of the GeneChip Array Station, please refer to the *GeneChip® Array Station User's Manual* (P/N 701859).

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## TRADEMARKS

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MEGAscript® is a trademark owned by Ambion, Inc.

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## CONTACT INFORMATION

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