GeneChip™ Expression 3' Amplification Reagents for IVT Labeling

Catalog Number 900449

Pub. No. 701486 **Rev.** 4

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**.

Introduction

The Applied Biosystems™ GeneChip™ Expression 3' Amplification Reagents for IVT Labeling is optimized specifically for producing amplified and biotinylated targets to hybridize to eukaryotic GeneChip™ brand arrays for expression analysis. Each kit contains sufficient reagents for completing 30 *in vitro* transcription reactions.

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 first reverse transcription reaction (described in the *GeneChip™ Expression Analysis Technical Manual*, available at **thermofisher.com**). Sufficient cDNA template can be obtained using one of the two protocols from high-quality starting materials:

- 1 to 15 µg of sample total RNA, or 0.2 to 2 µg of mRNA, using the One-Cycle cDNA Synthesis Procedure
- 10 ng to 100 ng of sample total RNA using the Two-Cycle cDNA Synthesis Procedure

Using this IVT Labeling Kit, over 30 µg of biotinylated cRNA targets are generated with the MEGAscript™ T7 Polymerase (**IVT Labeling Enzyme Mix**) in the presence of a mixture of the four natural ribonucleotides and one biotinconjugated nucleotide analog at optimized ratio (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 that is mediated by T7 RNA polymerase as a pseudouridine reagent. The biotinylated cRNA targets are then purified, fragmented, and hybridized to GeneChip expression arrays.

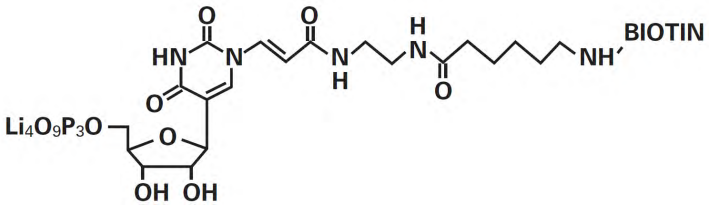

Figure 1 Structure of the biotin-conjugated nucleotide analog

A positive control, the **3′- Labeling Control**, is included in the kit and allows you to test the transcriptional activity of the kit components. Using 0.5 µg of the **3′- Labeling Control** as a template, routinely, over 30 µg of biotinylated IVT product is generated after 4 hours of incubation. The amplified, labeled product can be visually inspected on an agarose gel or other electrophoretic system, and a distinct band at approximately 2 Kb is typically observed.

Note: Hybridization cocktail preparation and fluidics scripts have been optimized specifically for using this IVT Labeling Kit. Follow the instructions for best results.

Components

Component	Volume
10X IVT Labeling Buffer	120 µL
IVT Labeling Enzyme Mix	120 µL
VT Labeling NTP Mix	360 µL
3′-Labeling Control (0.5 µg/µL)	10 µL
RNase-free Water	910 µL

Storage

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

Ordering information

Cat. No.	Product name	Description
900449	GeneChip™ Expression 3' Amplification Reagents for IVT Labeling	30 rxns
Other necessary reagents not provided in the kit		
900431	GeneChip™ One-Cycle cDNA Synthesis Kit	30 rxns
900432	GeneChip™ Two-Cycle cDNA Synthesis Kit	30 rxns
900371	GeneChip™ Sample Cleanup Module	30 rxns
900433	GeneChip™ Eukaryotic Poly-A RNA Control Kit	~ 100 rxns
900454	GeneChip™ Hybridization Control Kit	30 rxns
900457	GeneChip™ Hybridization Control Kit	150 rxns

IVT amplification and labeling procedure

Note: This kit is only used for the IVT labeling step for generating biotin-labeled cRNA. For the IVT amplification step using unlabeled ribonucleotides in the First Cycle of the Two-Cycle cDNA Synthesis Procedure, a separate kit is recommended (MEGAscript™ T7 Kit, Ambion, Inc.). Use only nuclease-free water, buffers, and pipette tips.

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

IMPORTANT! The Target Hybridizations and Array Washing protocols have been optimized specifically for this IVT Labeling Protocol. Closely follow the recommendations that are described in this section for maximum array performance.

1. Determine the amount of cDNA used for each IVT reaction following the cDNA cleanup step using the following table.

Starting material	Volume of cDNA to use in IVT
Total RNA	
10 to 100 ng	all (~12 µL)
1.0 to 8.0 µg	all (~12 µL)
8.1 to 15 µg	6 µL
mRNA	
0.2 to 2 µg	all (~12 µL)

2. Transfer the needed amount of template cDNA to RNase-free microcentrifuge tubes, then add the following reaction components in the order indicated in the following table. If more than one IVT reaction is to be performed, a master mix can be prepared by multiplying the reagent volumes by the number of reactions. Do not assemble the reaction on ice, as spermidine in the **10X IVT Labeling Buffer** can lead to precipitation of the template cDNA.

Reagent	Volume
Template cDNA[1]	Variable (see preceding table)
RNase-free Water	Variable (to give a final reaction volume of 40 µL)
10X IVT Labeling Buffer	4 µL
IVT Labeling NTP Mix	12 µL
IVT Labeling Enzyme Mix	4 µL
Total volume	**20 µL**

[1] 1 µg of the 3'-Labeling Control can be used instead of the template cDNA sample in this reaction as a positive control for the IVT components in the kit.

3. Carefully mix the reagents, then collect the mixture at the bottom of the tube by brief (5 seconds) microcentrifugation.

4. Incubate at 37°C for 16 hours. To prevent condensation that can result from water bath-style incubators, incubations are best performed in oven incubators for even temperature distribution, or in a thermal cycler.

   Note: Overnight IVT reaction time has been shown to maximize the labeled cRNA yield with high-quality array results. Alternatively, if a shorter incubation time (4 hours) is desired, 1 µL (200 units) of cloned T7 RNA polymerase (can be bought directly from Ambion, Cat. No. 2085) can be added to each reaction and has been shown to produce sufficient labeled cRNA yield in 4 hours. The two different incubation protocols generate comparable array results, and users are encouraged to select the procedure that best fits their experimental schedule and process flow.

5. Store labeled cRNA at –20°C, or –70°C if not purifying immediately. Alternatively, proceed to Cleanup and Quantification of Biotin-Labeled cRNA as described in the *GeneChip™ Expression Analysis Technical Manual*.

Eukaryotic target hybridization

1. Mix the following for each target, scaling up volumes for hybridization to multiple probe arrays.

   Note: A final concentration of 10% DMSO is added in the Hybridization Cocktail for optimal results.

   IMPORTANT! The frozen 20X Hybridization Control Stock must be heated to 65°C for 5 minutes to resuspend the cRNA before adding to the hybridization cocktail.

Component	49 and 64 Format	100 Format	400 and 169 Format	Final conc.
Fragmented cRNA[1]	15 µg	10 µg	5 µg	0.05 µg/µL
Control Oligonucleotide B2 (3 nM)	5 µL	3.3 µL	1.7 µL	50 psM
20X Eukaryotic Hybridization Controls (*bioB*, *bioC*, *bioD*, *cre*)	15 µL	10 µL	5 µL	1.5, 5, 25 and 100 pM, respectively
Herring Sperm DNA (10 mg/mL)	3 µL	2 µL	1 µL	0.1 mg/mL
Acetylated BSA (50 mg/mL)	3 µL	2 µL	1 µL	0.5 mg/mL
2X Hybridization Buffer	150 µL	100 µL	50 µL	1X
DMSO	30 µL	20 µL	10 µL	10%
H_2O	to final volume of 300 µL	to final volume of 200 µL	to final volume of 100 µL	
Final volume	**300 µL**	**200 µL**	**100 µL**	

[1] See Section 2, Chapter 1 in the *GeneChip™ Expression Analysis Technical Manual* for amount of adjusted fragmented cRNA to use when starting from total RNA.

Note: See specific probe array package inserts for information on array format.

2. Hybridize to expression probe arrays as described in the *GeneChip™ Expression Analysis Technical Manual*.

Wash and scan

1. Prepare the SAPE solution and Antibody solution as described in Section 2, Chapter 4 of the *GeneChip™ Expression Analysis Technical Manual* using the Antibody Amplification Stain Protocol for Eukaryotic Targets.

2. **For 49 Format and 64 Format arrays with 18 µm features**, use the existing EukGE-WS2v4 or EukGE-WS2v4_450 Fluidics Scripts.

   For 49 Format and 64 Format arrays with 11 µm features, download the new EukGE-WS2v5 Fluidics Scripts from **thermofisher.com**. Alternatively, modify the FlexEukGE-WS2v4 or FlexEukGE-WS2v4_450 Fluidics Scripts, and rename the scripts as EukGE-WS2v5 or EukGE-WS2v5_450.

   For 100 Format arrays, use the existing Midi-euk2 or Midi-euk2_450 Fluidics Scripts.

Step	49 and 64 Format: EukGE-WS2v5	100 Format (Midi): Midi_euk2[1]
Post Hyb Wash #1	10 cycles of 2 mixes/cycle with Wash Buffer A at 30°C	10 cycles of 2 mixes/cycle with Wash Buffer A at 30°C
Post Hyb Wash #2	6 cycles of 15 mixes/cycle with Wash Buffer B at 50°C	6 cycles of 15 mixes/cycle with Wash Buffer B at 50°C
Stain	Stain the probe array for 5 minutes in SAPE solution at 35°C	Stain the probe array for 5 minutes in SAPE solution at 35°C
Post Stain Wash	10 cycles of 4 mixes/cycle with Wash Buffer A at 30°C	10 cycles of 4 mixes/cycle with Wash Buffer A at 30°C
2nd Stain	Stain the probe array for 5 minutes in antibody solution at 35°C	Stain the probe array for 5 minutes in antibody solution at 35°C
3rd stain	Stain the probe array for 5 minutes in SAPE solution at 35°C	Stain the probe array for 5 minutes in SAPE solution at 35°C
Final Wash	15 cycles of 4 mixes/cycle with Wash Buffer A at 35°C. The holding temperature is 25°C	15 cycles of 4 mixes/cycle with Wash Buffer A at 35°C. The holding temperature is 25°C

[1] Midi_euk2_450 for the FS-450/250.

- Wash Buffer A = non-stringent wash buffer
- Wash Buffer B = stringent wash buffer

Functional testing and quality control

GeneChip™ Array Functional Testing: Each lot is functionally tested in the GeneChip™ expression assay following the recommended protocol.

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**.

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The information in this guide is subject to change without notice.

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Revision	Date	Description
4	01 August 2017	Updated document to current template.
3	Jan 2004	Baseline for revision history.