

## Affymetrix® Gene Profiling Reagent Kit

### Transcript Synthesis and Labeling Kit



#### Intended Use

For In Vitro Diagnostic Use

Affymetrix® Gene Profiling Reagents are intended for the preparation of labeled complementary RNA target from purified total RNA from fresh or frozen clinical tissue specimens for hybridization to Affymetrix GeneChip® microarrays and the measurement of fluorescence signals of labeled RNA target using the Affymetrix GeneChip® Microarray Instrumentation System.

Intended for use with separately FDA-cleared Affymetrix GeneChip microarray assays specifying the use of Affymetrix Gene Profiling Reagents.

#### Summary

Transcript Synthesis and Labeling Kit is optimized specifically for producing amplified and biotinylated complementary RNA (cRNA) targets to hybridize to arrays for expression analysis. The template DNA for the IVT reaction is typically double-stranded cDNA containing an RNA Polymerase promoter sequence. In target labeling experiments, the promoter sequence is incorporated into the cDNA template by using an Oligo(dT)-Promoter primer in the initial reverse transcription reaction. Sufficient cDNA template can be obtained using the protocols (described in the *Affymetrix Gene Profiling Reagents User Guide*) from high-quality starting materials. The labeled nucleotide analog is efficiently incorporated into the cRNA target during the *in vitro* transcription labeling reaction mediated by the RNA polymerase as a pseudouridine reagent. The biotinylated cRNA targets are then purified, fragmented, and hybridized to expression arrays.

#### Kit Components

The certificate of analysis is available on the Affymetrix website.

Component	P/N	Volume	Storage
<b>Transcript Synthesis and Labeling Kit A P/N 901293</b>			
1 <sup>st</sup> Strand Synthesis Buffer	901287	128 µL	2 to 8°C
2 <sup>nd</sup> Strand Synthesis Buffer	901288	576 µL	2 to 8°C
<i>In-Vitro</i> Transcription Buffer	901291	704 µL	2 to 8°C
Magnetic Beads	901290	3,456 µL	2 to 8°C
Beads Wash Buffer	901289	5,400 µL	2 to 8°C
Nuclease-free Water	901292	1,648 µL	2 to 8°C
<b>Transcript Synthesis and Labeling Kit B P/N 901298</b>			
1 <sup>st</sup> Strand Synthesis Enzyme Mix	901294	32 µL	-15 to -30°C
2 <sup>nd</sup> Strand Synthesis Enzyme Mix	901295	64 µL	-15 to -30°C
<i>In-Vitro</i> Transcription Enzyme Mix	901296	192 µL	-15 to -30°C
RNA Label	901297	64 µL	-15 to -30°C

#### Other Gene Profiling Reagents Not Provided With This Kit

Component	P/N
RNA Control Kit	901285
Transcript Detection Kit A, B, C	901299

#### Warnings and Precautions

1. For In Vitro Diagnostic Use.
2. Avoid microbial contamination, which may cause erroneous results.
3. All biological specimens and materials with which they come into contact should be handled as if capable of transmitting infection and disposed of with proper precautions in accordance with federal, state and local regulations. This includes adherence to the OSHA Bloodborne Pathogens Standard (29 CFR 1910.1030) for blood and other potentially infectious materials governed by this act. Never pipet by mouth. Avoid specimen contact with skin and mucous membranes.
4. Exercise standard precautions when obtaining, handling and disposing of potentially carcinogenic reagents.
5. Exercise care to avoid cross-contamination of samples during all steps of this procedure, as this may lead to erroneous results.
6. Use powder-free gloves whenever possible to minimize introduction of powder particles into sample or kit materials.
7. Once opened, this product is stable for 20 days when stored at the recommended storage temperature.
8. Performance of the kit has been shown to be unaffected for up to four freeze-thaw cycles.

#### Safety Information

A Material Safety Data Sheet(s) (MSDS) is available at [www.affymetrix.com](http://www.affymetrix.com). 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.

#### Indications of Instability or Deterioration

Inspect packages upon arrival. If the tamper-evident label is opened at the perforations, do not use the contents of the package. For customer service or technical support, please contact Affymetrix.

#### Workflow Procedures

##### Procedure 1: Preparation of 1<sup>st</sup> Strand cDNA Synthesis Reaction

**Note:** Mix the 1<sup>st</sup> Strand Synthesis Buffer and 1<sup>st</sup> Strand Synthesis Enzyme Mix prior to use by using gentle vortexing followed by a brief spin.

1. Prepare 1<sup>st</sup> Strand Master Mix at room temperature as described in Table 1.0. Make 15% extra 1<sup>st</sup> Strand Master Mix to ensure that sufficient volume is available for all wells. Mix by gentle vortexing followed by a brief spin.

**Table 1.0 1<sup>st</sup> Strand Master Mix Preparation**

Reagent	Volume/Reaction
1 <sup>st</sup> Strand Synthesis Buffer	4 µL
1 <sup>st</sup> Strand Synthesis Enzyme Mix	1 µL
Poly-A Control <sup>1</sup>	1 µL

<sup>1</sup> Poly-A Control must be diluted prior to use in this master mix. See the instructions accompanying the Poly-A Control Kit. Alternatively, detailed instructions are in the *Affymetrix Gene Profiling Assay Manual* which can be accessed at [www.affymetrix.com](http://www.affymetrix.com).

2. Transfer 6 µL 1<sup>st</sup> Strand Master Mix (room temperature), to the bottom of the appropriate wells of the 96-well plate sitting on a plastic rack. Discard any remaining 1<sup>st</sup> Strand Master Mix.
3. Add 4 µL of the total RNA samples (total of 100 ng to 1000 ng/rxn) into the appropriate wells of the 96-well plate, gently pipetting up and down 3 times to mix.
4. Cover the plate with aluminum adhesive foil and carefully seal tops with a brayer.
5. Centrifuge at 370 x g for no more than 10 seconds at room temperature to collect the solution at the bottom of the wells.
6. Remove the plate from the centrifuge and transfer to a thermal cycler, which is set at 42°C for 2 hour, 4°C for 10 minutes and 4°C hold for the 1<sup>st</sup> strand synthesis reaction. Cover the plate with a compression pad before closing the heated lid.
7. Remove the plate after completion of incubation at 42°C within 10 minutes of incubation at 4°C and centrifuge the plate at 370 x g for no more than 10 seconds at room temperature to collect the solution at the bottom of the wells. Remove the plate from the centrifuge and keep at room temperature.
8. Immediately proceed to the preparation of 2<sup>nd</sup> Strand cDNA Synthesis Reaction.

## Procedure 2: Preparation of 2<sup>nd</sup> Strand cDNA Synthesis Reaction

**Note:** Mix the 2<sup>nd</sup> Strand Synthesis Buffer and 2<sup>nd</sup> Strand Synthesis Enzyme Mix prior to use by using gentle vortexing followed by a brief spin.

1. Prepare 2<sup>nd</sup> Strand Master Mix in a 1.5 mL nuclease-free tube at room temperature as described in Table 2.0. Make 10% extra 2<sup>nd</sup> Strand Master Mix to ensure that sufficient volume is available for all wells. Mix by gentle vortexing followed by a brief spin.

**Table 2.0 2<sup>nd</sup> Strand Master Mix Preparation**

Reagent	Volume/Reaction
2 <sup>nd</sup> Strand Synthesis Buffer	18 µL
2 <sup>nd</sup> Strand Synthesis Enzyme Mix	2 µL

2. Carefully remove the aluminum adhesive foil from the plate sitting on a plastic rack.
3. Transfer 20 µL of 2<sup>nd</sup> Strand Master Mix into the side wall of the appropriate wells of the 96-well plate at room temperature.
4. Cover the plate with aluminum adhesive foil and carefully seal the top with a brayer. Discard any remaining 2<sup>nd</sup> Strand Master Mix.
5. Centrifuge at 370 x g for no more than 10 seconds at room temperature to collect the solution at the bottom of the wells.
6. Remove the plate from the centrifuge and transfer to a thermal cycler, which is set at 16°C for 1 hour, 4°C for 10 minutes and 4°C hold for the 2<sup>nd</sup> strand synthesis reaction.

**Important:** Do not cover the plate with the heated lid during 16°C incubation.

7. Remove the plate after the completion of 16°C incubation within 10 minutes of incubation at 4°C and centrifuge the plate at 370 x g for no more than 10 seconds at room temperature to collect the solution at the bottom of the wells. Remove the plate from the centrifuge and keep at room temperature.
8. Immediately proceed to the Preparation of the In Vitro Transcription Reaction.

## Procedure 3: Preparation of the In Vitro Transcription (IVT) Reaction

**Note:** Mix the In-Vitro Transcription Buffer, In-Vitro Transcription Enzyme Mix, and RNA Label prior to use by using gentle vortexing followed by a brief spin.

1. Prepare IVT Master Mix at room temperature as described in Table 3.0. Make 10% extra IVT Master Mix to ensure that sufficient volume is available for all wells. Mix by gentle vortexing followed by a brief spin.

**Table 3.0 IVT Master Mix Preparation**

Reagent	Volume/Reaction
In-Vitro Transcription Buffer	22 µL
RNA Label	2 µL
In-Vitro Transcription Enzyme Mix	6 µL

2. Carefully remove the aluminum adhesive foil from the plate sitting on a plastic rack.
3. Transfer 30 µL of IVT Master Mix into the side wall of the appropriate wells of the 96-well plate containing the 30 µL of 2<sup>nd</sup> strand reaction.
4. Cover the plate with aluminum adhesive foil and carefully seal tops with a brayer.
5. Centrifuge at 370 x g for no more than 10 seconds at room temperature to collect the solution at the bottom of the wells.
6. Remove the plate from the centrifuge and transfer to a thermal cycler, which is set at 40°C for 16 hours\*, 4°C hold for the IVT reaction. Cover the plate with a compression pad before closing the heated lid.

\*Incubation time was optimized using 100 ng of commercially available total RNA extracted from HeLa cells. The time should be adjusted based on the identity, quality, and starting amount of total RNA used in the assay.

7. Remove the plate after 40°C incubation (when the thermal cycler display shows a temperature of 4°C), centrifuge the plate at 370 x g for no more than 10 seconds at room temperature to collect the solution at the bottom of the wells. Remove plate from the centrifuge and keep at room temperature. Proceed to the Purification of the cRNA.

## Procedure 4: Purification of the cRNA from the In Vitro Transcription (IVT) Reaction

**Important:** Before starting the purification, transfer Nuclease-free water into a 1.5 mL tube. Place the Nuclease-free water at 60°C for at least 10 minutes on the heat block, leaving the Nuclease-free water at 60°C until use.

**Important:** Before the first use of the Beads Wash Buffer from the kit, add 12.6 mL of 100% ethanol to Beads Wash Buffer and make a checkmark in the check box, initial and date.

1. Gently shake the bottle of magnetic beads to resuspend the particles that may have settled.
2. Add 108 µL of Magnetic Beads solution into each IVT reaction mixture. Mix by gently pipetting up and down.
3. Transfer samples from the 96-well plate to the U-bottom 96-well plate.
4. Transfer the U-bottom plate to the plate shaker and shake at medium speed for 2 minutes at room temperature.
5. Place the plate on the magnetic stand and allow the beads to pellet for 5 to 10 minutes until the solution is clear and the beads form a pellet against the magnet.
6. Once the mixture is transparent, using a multi-channel pipette remove and discard the supernatant without disturbing the beads.
7. Leave the plate on the magnetic stand, and wash each sample with 200 µL of Bead Wash Buffer two times, removing all supernatant after each wash. Incubate for 25-35 seconds at room temperature during each wash.
8. Let the plate air-dry for 5 to 7 minutes sitting on the magnetic stand.
9. Remove the plate from the magnetic stand, then add 30 µL of Nuclease-free Water heated at 60°C to the side wall of each sample well using a repeater pipette.

**Important:** Due to the high temperature of the Nuclease-free Water, when using the repeater pipette, allow for two extra 30 µL volumes: discard the first 30 µL before adding to the samples and do not use the last 30 µL of the elution solution. It is important to follow the recommendation described here to ensure that correct volumes are dispensed to each sample.

10. Transfer the U-bottom plate to a shaker. Shake at high speed for one minute. A micropipettor may be used to fully disrupt the pellet if it does not go into solution.
11. Move the plate to the magnetic stand and allow beads to settle for 3 to 4 minutes.
12. Once the solution is transparent and the beads have settled, remove 30 µL of the supernatant without disturbing the pellet. Transfer the supernatant containing the eluted cRNA to a new 96-well plate or individual tubes.

Detailed steps and instructions for above procedures are in the *Affymetrix Gene Profiling Reagents User Guide* which can be accessed at [www.affymetrix.com](http://www.affymetrix.com).

## Limitations of the Procedure

Proper storage and handling of reagents and samples is essential for the performance. Do not store Transcript Synthesis and Labeling Kit B in a frost-free freezer. All laboratory equipment used to prepare the target during this procedure should be calibrated and maintained to ensure accuracy. Incorrect measurement of reagents may affect the outcome of the procedure.

## Patents

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## Symbol Table

Table below shows the legend of the graphic symbols used for Affymetrix product label, package inserts and User Guide.

**Table 4.0 Graphic Symbols for use in Labeling**

Symbol / Letters	Statement
	Part/Catalog Number
	Lot Number
	Expiration Date YYYY-MM Kit will expire on the last day of the month.
	Temperature Limitation
	Contains Sufficient for < n > Tests
Xi	Irritant
	Hazards
	Consult Instructions for Use
	Manufacturer
	In Vitro Diagnostic Medical Device
	European Conformity
	Authorized Representative in the European Union

Translated versions of this package insert are available on the Affymetrix website.

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