

Affymetrix® Gene Profiling Reagent Kit

RNA Control 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

The RNA Control Kit is designed specifically to provide exogenous positive controls to monitor the entire eukaryotic target synthesis and labeling process. Each eukaryotic probe array contains probe sets for several *B. subtilis* genes that are absent in the samples analyzed (*lys*, *phe*, and *dap*). RNA Control Kit contains *in vitro* synthesized, polyadenylated transcripts for these *B. subtilis* genes that are pre-mixed at staggered concentrations to allow probe array users to assess the overall success of the assay. The concentrated Poly-A Control can be diluted with the Dilution Buffer (supplied as part of the kit) and spiked directly into the total RNA samples to achieve the final concentrations (referred to as a ratio of copy number) summarized below:

Poly-A Control	Final Concentration
<i>lys</i>	1:100,000
<i>phe</i>	1:50,000
<i>dap</i>	1:6,667

The controls are then amplified and labeled together with the samples. Examining the hybridization intensities of these controls on arrays helps users monitor the amplification and labeling process independently from the quality of the starting total RNA samples. Detailed procedure for using this kit is described in the *Affymetrix Gene Profiling Reagents User Guide*.

Kit Components

The certificate of analysis is available on the Affymetrix website.

Component	P/N	Volume	Storage
RNA Control Kit P/N 901285			
Poly-A Control	901284	16 µL	-15 to -30°C
Dilution Buffer	901283	3.8 mL	-15 to -30°C

Other Gene Profiling Reagents Not Provided With This Kit

Component	P/N
Transcript Synthesis and Labeling Kit A, B	901286
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 30 days when stored at the recommended storage temperature.
8. Performance of the kit has been shown to be unaffected for up to eight freeze-thaw cycles.

Safety Information

A Material Safety Data Sheet(s) (MSDS) is available at 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

The Poly-A Control and Dilution Buffer are provided with the kit to prepare the appropriate serial dilutions based on starting amount of total RNA (Table 1.0). This is a guideline when 100, 200, 500, or 1000 ng of total RNA is used as starting material. For other intermediate starting sample amounts, calculations are needed in order to perform the appropriate dilutions to arrive at the same proportionate final concentration of the spike-in controls in the samples.

Detailed steps and instructions are in the *Affymetrix Gene Profiling Reagents User Guide* which can be accessed at www.affymetrix.com.

Table 1.0 Serial Dilutions of Poly-A Control

Starting Amount of Total RNA	Serial Dilutions				Volume to Add to Total RNA
	First	Second	Third	Fourth	
100 ng	1:20	1:50	1:50	1:5	1 µL
200 ng	1:20	1:50	1:50	1:2.5	1 µL
500 ng	1:20	1:50	1:50		1 µL
1000 ng	1:20	1:50	1:25		1 µL

For example, to prepare the Poly-A dilutions for 200 ng of total RNA:

1. Label 4 non-stick RNase-free microcentrifuge tubes with 1st, 2nd, 3rd and 4th Dilution.
2. After the Dilution Buffer is thawed at room temperature, pipette 38 µL of the Dilution Buffer in the 1st dilution tube, 98 µL of the Dilution Buffer in the 2nd and 3rd dilution tubes, and 6 µL of Dilution Buffer to the 4th dilution tube. Return Dilution Buffer to the freezer. Keep tubes at room temperature.
3. Thaw the Poly-A Control at room temperature. After it has thawed, mix by flicking the tube and spin down to collect the liquid at the bottom of the tube. Immediately proceed to Step 4.
4. Add 2 µL of the Poly-A Control solution to the 38 µL of Dilution Buffer in the tube labeled 1st Dilution (1:20). Return the Poly-A Control to the freezer.
5. Mix thoroughly by flicking the tube and spin down to collect the liquid at the bottom of the tube.
6. Add 2 µL of the 1st Dilution to 98 µL of Dilution Buffer in the tube labeled 2nd Dilution (1:50).
7. Mix thoroughly by flicking and spin down to collect the liquid at the bottom of the tube.
8. Add 2 µL of the 2nd Dilution to 98 µL of Dilution Buffer in the tube labeled 3rd Dilution (1:50).

9. Mix thoroughly by flicking and spin down to collect the liquid at the bottom of the tube.
10. Add 4 μ L of the 3rd Dilution to 6 μ L of Dilution Buffer in the tube labeled 4th Dilution (1:2.5).
11. Mix thoroughly by flicking and spin down to collect the liquid at the bottom of the tube.
12. Use the 4th Dilution to prepare the solution described in Preparation of 1st Strand cDNA Synthesis Reaction

Detailed steps and instructions for above procedures are in the *Affymetrix Gene Profiling Reagents User Guide* which can be accessed at www.affymetrix.com.

Limitations of the Procedure

Proper storage and handling of reagents and samples is essential for the performance. Do not store RNA Control Kit 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.

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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 2.0 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
	<i>In vitro</i> Diagnostic Medical Device
	European Conformity
	Authorized Representative in the European Union

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

Contact Information

 Affymetrix, Inc.,
3420 Central Expressway,
Santa Clara, CA 95051 USA

Manufactured by Asuragen® for Affymetrix, Inc.

 Emergo Europe
Molenstraat 15
2513 BH, The Hague
The Netherlands
Phone: +31.70.345.8570
Fax: +31.70.346.7299

For technical support, please contact Affymetrix at the appropriate e-mail address or phone number below.

Affymetrix, Inc.

3420 Central Expressway
Santa Clara, CA 95051 USA
E-mail: support@affymetrix.com
Tel: 1-888-362-2447 (1-888-DNA-CHIP)
Fax: 1-408-731-5441

Affymetrix UK Ltd

Voyager, Mercury Park,
Wycombe Lane, Wooburn Green,
High Wycombe HP10 0HH
United Kingdom
E-mail: supporteurope@affymetrix.com
Tel: +44 (0) 1628 552550
Fax: +44 (0) 1628 552585
www.affymetrix.com