

Affymetrix® Gene Profiling Reagent Kit

Transcript Detection 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 Detection Kit is optimized for the fragmentation of the labeled cRNA target, hybridization, staining and washing of arrays for expression analysis. A hybridization cocktail is prepared including the labeled, fragmented target and hybridization controls. The target is then hybridized to the array. Immediately following hybridization, the array is washed and stained with streptavidin phycoerythrin conjugate followed by scanning.

Kit Components

The certificate of analysis is available on the Affymetrix website.

Component	P/N	Volume	Storage
Transcript Detection Kit A P/N 901307			
Hybridization Module			
■ Pre-Hybridization Mix	901304	6.4 mL	2 to 8°C
■ 2x Hybridization Mix	901300	4.0 mL	2 to 8°C
■ DMSO	901303	0.8 mL	2 to 8°C
■ Nuclease-free Water	901332	5.0 mL	2 to 8°C
■ 5x Fragmentation Buffer	901301	192 µL	2 to 8°C
Stain Module			
■ Stain Cocktail 1	901305	19.2 mL	2 to 8°C
■ Stain Cocktail 2	901306	19.2 mL	2 to 8°C
■ Array Holding Buffer (2 units)	901302	30.0 mL	2 to 8°C
Transcript Detection Kit B P/N 901310			
Wash Buffer A (3 units)	901308	860 mL	2 to 8°C
Wash Buffer B	901309	640 mL	2 to 8°C
Transcript Detection Kit C P/N 901312			
Oligo B2	901313	134.4 µL	-15 to -30°C
20x Hybridization Control	901311	400 µL	-15 to -30°C

Other Gene Profiling Reagents Not Provided With This Kit

Product	Affymetrix P/N
RNA Control Kit	901285
Transcript Synthesis and Labeling Kit A, B	901286

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 open, this product is stable for 30 days when stored at the recommended storage temperature.
8. Performance of DMSO, Oligo B2, and 20X Hybridization Control have 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.

Caution:

DMSO (Dimethyl Sulfoxide): Combustible Liquid, Irritant. Readily absorbed through the skin [CAS# 67-68-5].

The Pre-Hybridization Mix, 2X Hybridization Mix, 5X Fragmentation Buffer, Stain Cocktail 1, Stain Cocktail 2, Array Holding Buffer, Wash Buffer A and Wash Buffer B contain ≤ 0.10% Sodium Azide [CAS# 26628-22-8].

Indications of Instability or Deterioration

Inspect packages upon arrival. Do not use the reagents if the reagent vials are opened or punctured. For customer service or technical support, please contact Affymetrix.

Workflow Procedures

Procedure 1: Preparation of the cRNA Fragmentation Reaction

1. Mix the 5X Fragmentation Buffer by gentle vortexing, and briefly spin down to collect the contents at the bottom of the tube.
2. Table 1.0 shows the fragmentation reaction mix for cRNA samples at a final concentration of 0.5 µg/µL. Using the adjusted cRNA yield calculate the volume of cRNA required to add 15 µg to the fragmentation reaction.

Table 1.0 Sample Fragmentation Reaction Preparation

Component	Amount or Volume
cRNA	15 µg
5X Fragmentation Buffer	6 µL
Nuclease-free Water (variable)	To 30 µL final volume
Total Volume	30 µL

3. Set up the reaction in a 0.2 mL strip tube.
4. Mix by gentle vortexing, and briefly spin down to collect the contents at the bottom of the tube.
5. Transfer the strip tubes to a thermal cycler set at 94°C for 35 minutes and hold at 4°C. Cover with the heated lid. Start the method and confirm the appropriate volume of the reaction for this step: 30 µL.
6. Proceed to Procedure 2.

Procedure 2: Hybridization

This section describes preparation for a 49-format array.

Note: DMSO will solidify when stored at 4°C. Please ensure that the reagent is completely thawed prior to use.

Note: Set the temperature of dry-heatblocks to 45°C, 65°C and 99°C.

1. Remove Oligo B2 and 20X Hybridization Control from the freezer and thaw at room temperature.

IMPORTANT: It is imperative that stocks of 20X Hybridization Control are heated to 65°C for 5 minutes to completely resuspend the cRNA before aliquoting.

2. Prepare Hybridization Master Mix at room temperature for one or multiple probe arrays as outlined in Table 2.0.

Table 2.0 Hybridization Master Mix

Component	Working Master Mix Volumes Sufficient for 1 Probe Array (V)	Working Master Mix Volumes Sufficient for 1 Probe Array x 1.10 (V x 1.10)	Desired Number of Probe Arrays (R)	Total Volume Required (V x 1.10) x R	Final Dilution/Concentration
Oligo B2 (3 nM)	4.2 µL	4.62 µL			50 pM
20X Hybridization Control (<i>bioB</i> , <i>bioC</i> , <i>bioD</i> , <i>cre</i>)	12.5 µL	13.75 µL			1.5, 5, 25 and 100 pM respectively
2X Hybridization Mix	125 µL	137.5 µL			1X
DMSO	25 µL	27.5 µL			10%
Nuclease-free Water	58.3 µL	64.13 µL			
Total Volume	225.0 µL	247.5 µL			

- a. Aliquot 225 µL of the Hybridization Master Mix into a Nuclease-free 1.5 mL tube.
- b. Add 25 µL of fragmented cRNA from Procedure 1.5 above to prepare the Hybridization Cocktail for one probe array. Final concentration of cRNA in the Hybridization Cocktail is 0.05 µg/µL

3. Equilibrate probe array to room temperature immediately before use.

Note: It is important to allow the probe arrays to equilibrate to room temperature completely. Specifically, if the rubber septa are not equilibrated to room temperature, they may be prone to cracking, which can lead to leaks.

4. Heat the hybridization cocktail to 99°C for 5 minutes in a heatblock.
5. Meanwhile wet the probe array with 200 µL of Pre-Hybridization Mix by filling it through one of the septa.
6. Incubate the probe array filled with Pre-Hybridization Mix at 45°C for 10 minutes at 60 rpm in the hybridization oven.
7. Transfer the hybridization cocktail that has been heated at 99°C, in Step 4 above, to a 45°C heatblock for 5 minutes.
8. Spin the hybridization cocktail at maximum speed in a microcentrifuge for 5 minutes at room temperature to collect any insoluble material from the hybridization mixture.
9. Remove the probe array from the hybridization oven. Vent the probe array with a clean pipette tip and extract the Pre-Hybridization Mix from the probe array with a micropipettor. Refill the array with the 200 µL of the clarified hybridization cocktail, avoiding any insoluble matter at the bottom of the tube.
10. Carefully apply one adhesive label dot to each of the two septa. Press to ensure that the spots remain flat. If the adhesive label dots do not apply smoothly, that is, if you observe bumps, bubbles, tears, or curled edges, do not attempt to smooth out the dot. Remove the dot and apply a new one.
11. Place probe array into the hybridization oven, set to 45°C. Rotate at 60 rpm.
12. Hybridize for 17 ± 1 hours.
13. During the latter part of the 17-hour hybridization, proceed to Step 3 to prepare reagents for the washing and staining steps required immediately after completion of hybridization.

Note: For detailed information on using the Affymetrix Hybridization Oven 645, see the *Affymetrix Hybridization Oven 645 User's Guide*, P/N 08-0255 and the *Affymetrix Hybridization Oven 645 Quick Reference Card*, P/N 08-0256.

Procedure 3: Probe Array Wash and Stain

After 17 ± 1 hours of hybridization remove the array from the hybridization oven. Remove the adhesive label dots and vent the probe array by inserting a clean pipette tip into one of the septa, and extract the hybridization cocktail with a pipettor through the remaining septum. Refill the probe array completely with 250 µL of Wash Buffer A.

Note: If necessary, at this point the probe array can be stored at 4°C, protected from light, for up to 3 hours before proceeding with washing and staining. Equilibrate to room temperature before washing and staining.

Preparing the Stain Reagents

Prepare the following reagents. Volumes given are sufficient for 1 probe array.

1. Remove Stain Cocktail 1, Stain Cocktail 2, and Array Holding Buffer from the storage at 2 to 8°C.
2. Gently tap the bottles to mix well.
3. Aliquot the following reagents:
4. 600 µL of Stain Cocktail 1 into a 1.5 mL amber microcentrifuge vial.
5. 600 µL of Stain Cocktail 2 into a 1.5 mL (clear) microcentrifuge vial.
6. 800 µL of Array Holding Buffer into a 1.5 mL (clear) microcentrifuge vial.
7. Spin down all vials to remove the presence of any air bubbles.

Note: Stain Cocktail 1 is light-sensitive. Please be sure to use amber microcentrifuge vials when aliquoting.

Setting Up the Fluidics Station

Follow the instruction in *Gene Profiling Reagents User Guide*

Using the Fluidics Station

Once you have set up and primed the fluidics station, you can now proceed to use the fluidics station in your assay.

1. Click the Fluidics button from the left workflow panel. The Fluidics Worklist appears.
2. If you are entering the information manually, select a test request record with the desired Array ID and enter the fluidics station number in the Station # field and module number in the Module # field for the test request.
3. Insert the appropriate probe array into the designated module of the fluidics station while the cartridge lever is in the down, or eject position. When finished, verify that the cartridge lever is returned to the up, or engaged, position.
4. Remove any microcentrifuge vial remaining in the sample holder of the fluidics station module(s) being used.
5. If you are using a barcode reader, scan each array then immediately scan the fluidics station module that will process the array. The Array ID on the array will identify the proper test request registered to that Array ID. The status of the test request will change to Ready.
6. Select the test requests in AMDS.
7. Click the Start button on the toolbar of the Fluidics Worklist panel.
8. Follow the instructions on the LCD window on the fluidics station by placing the three experiment sample vials (the microcentrifuge vials) into the sample holders 1, 2, and 3 on the fluidics station.
 - a. Place one vial containing 600 µL Stain Cocktail 1 in sample holder 1.
 - b. Place one vial containing 600 µL Stain Cocktail 2 in sample holder 2.
 - c. Place one vial containing 800 µL of Array Holding Buffer in sample holder 3.
 - d. Press down on the needle lever to snap needles into position and to start the run.

As the run begins, the Fluidics Station dialog box at the workstation terminal and the LCD window display the status of the washing and staining as the protocol progresses.

9. When the protocol is complete, the LCD window on the fluidics station displays the message EJECT & INSPECT CARTRIDGE.
10. Remove the probe arrays from the fluidics station modules by first pressing down the cartridge lever to the eject position.
11. Check the probe array window for large bubbles or air pockets.
 - If the probe array has no large bubbles, it is ready to scan. Pull up on the cartridge lever to engage washblock and proceed to Step 4: Probe Array Scan.
 - If bubbles are present, do the following:

Return the probe array to the probe array holder. Add 800 µL of Array Holding Buffer to vial #3 in sample holder 3. Follow instructions on the

LCD window. Engage the washblock by gently pushing up on the cartridge lever to the engaged, or closed, position.

The fluidics station will drain the probe array and then fill it with a fresh volume of Array Holding Buffer. When it is finished, the LCD window will display EJECT & INSPECT CARTRIDGE. Again, remove the probe array and inspect it for bubbles. If no bubbles are present, it is ready to scan. Pull up on the lever to close the washblock and proceed to Procedure 4: Probe Array Scan.

Note: If the attempt to fill the probe array without bubbles is unsuccessful, the array should be filled manually with Array Holding Buffer using a micropipette. Excessive washing will result in a loss of signal intensity.

12. If you do not scan the arrays right away, keep the probe arrays at 4 °C and in the dark until ready for scanning.

Procedure 4: Probe Array Scan

Follow the instruction in *Gene Profiling Reagents User Guide*.

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.

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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 3.0 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.

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