

Standardized Assays and Reagents for GeneChip® Expression Analysis

blood protocols

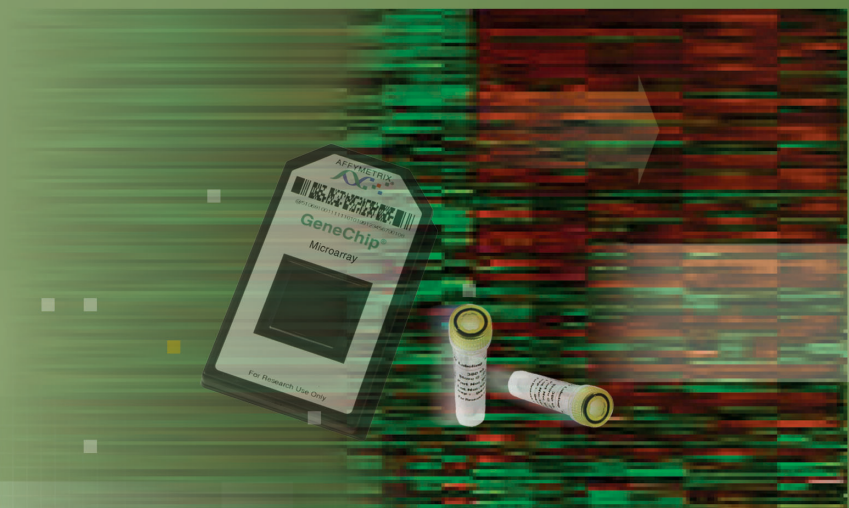
system solution

validated

integrated

small sample

standardization



The central image features a GeneChip Microarray in its packaging, two small reagent vials, and a heatmap background with a large green arrow pointing right. The GeneChip packaging is labeled 'AFFYMETRIX', 'GeneChip', and 'Microarray'. The vials are labeled 'Affymetrix' and 'GeneChip'. The heatmap background shows a grid of red and green spots, with a large green arrow pointing right. The text 'blood protocols', 'system solution', 'validated', 'integrated', 'small sample', and 'standardization' is overlaid on the image.





Tools to take you as far as your vision.™



■ ■ Ensure Robust, Reproducible Results with Standardized GeneChip® Assays and Reagents

Standardized, reliable assays and reagents are integral components of the GeneChip® System solution, allowing researchers to obtain high-quality data and reproducible results on GeneChip brand arrays. These robust products are convenient and easy to use for simplified sample and target preparation.

Optimized assays are specifically designed for a wide range of sample types and applications.

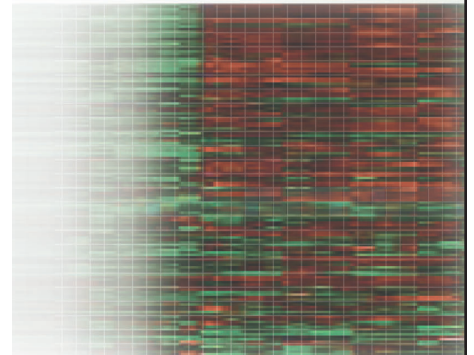
- Labeling assays uniquely complement Affymetrix' sequence and probe selection strategies for whole-genome expression analysis with optimal performance.
- Several target labeling and sample preparation assays are available, particularly suited for working with diverse starting sample types, providing ease of use and confidence for GeneChip expression analysis.

Validated reagents are quality tested on GeneChip® expression arrays.

- All reagents are assured for success and consistency on GeneChip arrays.
- All arrays include built-in controls. The corresponding set of control reagents is available to monitor the entire GeneChip expression experiment, from target preparation to array hybridization.

Standardized single-color labeling for ultimate comparability.

- Single-color labeling facilitates *in silico* comparison of expression data across any number of arrays, experiments, and labs. Researchers can now compare, publish, and share data without limitation.



■ ■ Eukaryotic Target Labeling Assays – Unmatched Scalability and Reproducibility

■ **Tailored assays for standard or limited starting materials** – Two eukaryotic GeneChip® target labeling protocols are available to ensure successful expression analysis. Scientists can select the procedure that best matches their sample requirements.

■ **Linear amplification and labeling strategy for reliable results** – Both assays utilize an Oligo(dT)-primed, *in vitro* transcription-based linear amplification strategy to generate high-yield, biotinylated targets from the 3'-end. This ensures compatibility with the current array design strategy, which targets sequences in the region of 600 bases proximal to the 3'-end of each transcript. One-Cycle and Two-Cycle Target Labeling Assays were extensively tested on GeneChip arrays to reliably detect transcript expression levels.

■ **Specialized solutions for unique sample types** – Challenging sample types occasionally require additional processing prior to target labeling. For example, to assist researchers working with blood samples, Affymetrix performed studies to evaluate how several blood processing and RNA isolation procedures might influence the GeneChip array data. As a result, researchers now have access to sample data on GeneChip arrays, and can choose the most appropriate protocol for their own unique research requirements. More detailed information on using blood for GeneChip analysis can be found in the Technical Notes entitled “*An Analysis of Blood Processing Methods to Prepare Samples for GeneChip® Expression Profiling*” and “*Globin Reduction Protocol: A Method for Processing Whole Blood RNA Samples for Improved Array Results*”.

Starting Material		Protocol
Total RNA	mRNA	
1 µg – 15 µg	0.2 µg – 2 µg	One-Cycle Target Labeling
10 ng – 100 ng	N/A	Two-Cycle Target Labeling



GeneChip® Eukaryotic Target Labeling Assays for Expression Analysis

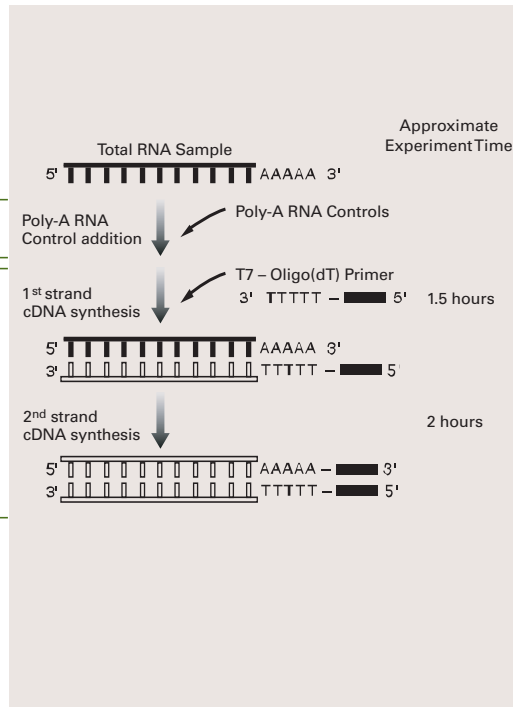
One-Cycle Target Labeling

(for 1-15 µg total RNA or 0.2-2 µg mRNA)

Products Used

Poly-A RNA Control Kit

One-Cycle cDNA Synthesis Kit



Two-Cycle Target Labeling

(for 10-100 ng total RNA)

Approximate Experiment Time

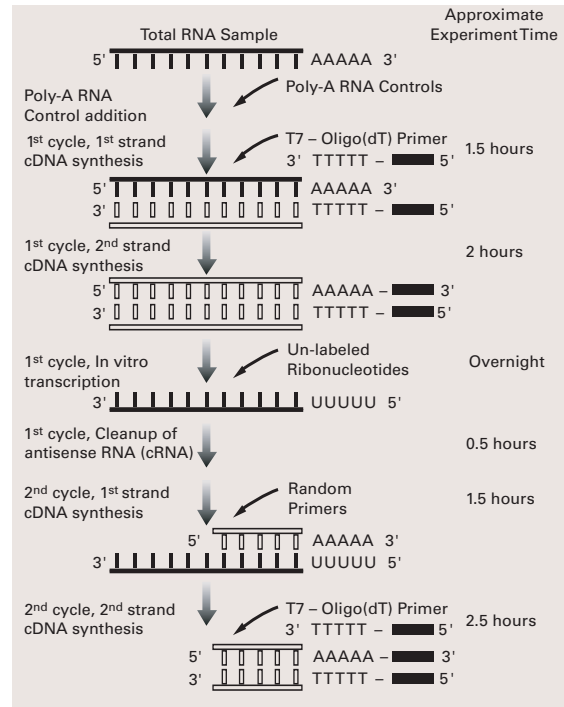
Products Used

Poly-A RNA Control Kit

Two-Cycle cDNA Synthesis Kit

Sample Cleanup Module

Two-Cycle cDNA Synthesis Kit



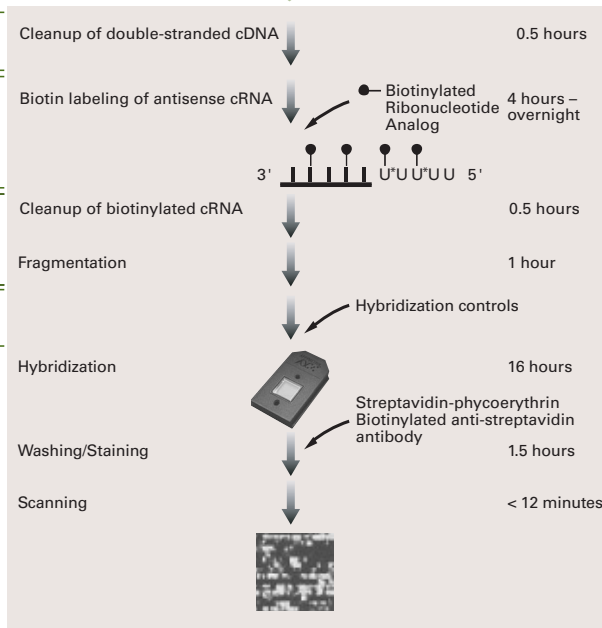
Products Used

Sample Cleanup Module

IVT Labeling Kit

Sample Cleanup Module

Hybridization Control Kit



Legend

- TTTTT RNA
- TTTTT DNA
- T7 promoter
- Biotin
- U* Pseudouridine



■ ■ Labeling Reagents

One-Cycle cDNA Synthesis Kit

- Provides all necessary reagents in one convenient kit for double-stranded cDNA synthesis from 1 to 15 µg of total RNA or 0.2 to 2 µg of mRNA
- Manufactured by Invitrogen for Affymetrix, uniquely configured and tested for GeneChip target labeling
- Optimized for the GeneChip platform and based on proven, robust protocol
- Provides consistent performance with SuperScript™ II for reverse transcription
- Limited license to Van Gelder/Eberwine patents via Incyte

Two-Cycle cDNA Synthesis Kit

- Provides all necessary reagents in one convenient kit for performing two cycles of double-stranded cDNA synthesis from 10 to 100 ng of total RNA
- Manufactured by Invitrogen for Affymetrix, uniquely configured and tested for GeneChip target labeling
- Designed for applications with limited starting materials
- Optimized for the GeneChip platform and based on a proven, robust, and streamlined protocol that completes target labeling in only 2½ days
- Consistent performance with SuperScript™ II for reverse transcription
- Limited license to Van Gelder/Eberwine patents via Incyte

Sample Cleanup Module

- Developed and manufactured by QIAGEN® specifically for GeneChip arrays
- Conveniently packaged to support three steps within the target labeling assay: cDNA cleanup, IVT cRNA cleanup, and cRNA fragmentation
- Fast, easy-to-use, and reliable spin columns for both cleanup procedures
- Optimized elution volume compatible with the assay flow, eliminating the need to concentrate samples
- Removes a key bottleneck for assay automation

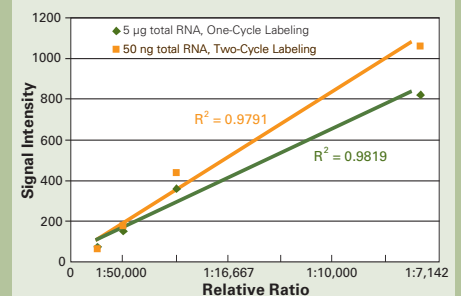
■ ■ Control Reagents

Eukaryotic Poly-A RNA Control Kit

- Conveniently monitors the entire target labeling process to evaluate assay sensitivity, consistency, and dynamic range with an easy-to-use reagent
- Contains four exogenous, pre-mixed, polyadenylated prokaryotic controls (*lys*, *phe*, *thr*, and *dap*) that are spiked directly into RNA samples before target labeling. Their resultant signal intensities on GeneChip arrays serve as sensitive indicators of target preparation and the labeling reaction efficiency, independent from starting sample quality
- Designed and quality controlled specifically for GeneChip brand arrays and protocols for use in both the One-Cycle and Two-Cycle Target Labeling Assays

Poly-A RNA Controls

(<i>B. subtilis</i>)	Final Concentration in Samples
<i>lys</i>	1: 100,000
<i>phe</i>	1: 50,000
<i>thr</i>	1: 25,000
<i>dap</i>	1: 7,500



T7-Oligo(dT) Promoter Primer Kit

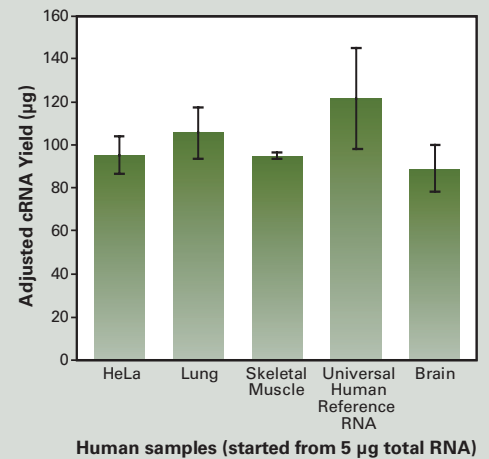
- Sequence:
5' – GGCCAGTGAATTGTAATACGACT
CACTATAGGGAGGCGG – (dT)₂₄ – 3'
- Consistent batch-to-batch concentration and purity
- Ability to withstand at least 25 freeze-thaw cycles without compromising performance
- Included in the One-Cycle and Two-Cycle cDNA Synthesis Kits

IVT Labeling Kit

- Created for *in vitro* transcription (IVT) amplification and labeling using double-stranded cDNA containing the T7 promoter sequence as template, from either the One-Cycle or Two-Cycle Target Labeling Assays
- Utilizes a proprietary synthetic biotinylated nucleotide analog (pseudouridine base) reagent and *in vitro* transcription with MEGAscript® reagents from Ambion for high cRNA yield and uniform incorporation efficiency
- Designed and quality tested specifically for GeneChip brand arrays for consistent performance
- Includes an independent double-stranded positive control template to test the transcriptional activity of the kit components

Biotin-labeled cRNA yield

Average yields and standard deviations from independent labeling reactions. Consistent high yields are obtained from all tissues.



Human samples (started from 5 µg total RNA)

Hybridization Controls

- High-quality controls for monitoring array hybridization, washing, and staining for reproducible results
- 20X Eukaryotic Hybridization Controls composed of a mixture of biotinylated and fragmented cRNA of *bioB*, *bioC*, and *bioD* from *E. coli*, and *cre* from P1 bacteriophage in staggered concentrations. The premixed controls are ready to be added directly to the hybridization cocktail. Probes detecting these controls are present on all GeneChip eukaryotic expression probe arrays
- Also includes Control Oligo B2 (3 nM) to provide control and alignment signals for the image analysis

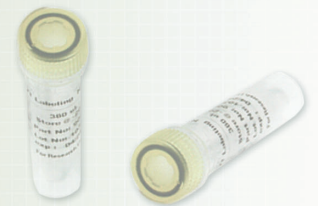


Prokaryotic Target Labeling Assay: Consistent Strategy and Quality

A single-color labeling strategy is also utilized in the prokaryotic target preparation.

cDNA labeling for antisense prokaryotic arrays. Since prokaryotic mRNA lacks the poly-A tail, a random priming and end-labeling cDNA protocol is utilized. Following cDNA synthesis with random hexamers, the cDNA products are fragmented by DNase I and labeled with Bio-ddUTP.

For a complete listing of all reagents required for the assay, please refer to the *GeneChip® Expression Analysis Technical Manual*.



Support Information

For additional information on GeneChip assays for expression analysis, reference the following documents on www.affymetrix.com:

- *GeneChip® Expression Analysis Technical Manual*
- *An Analysis of Blood Processing Methods to Prepare Samples for GeneChip® Expression Profiling*
- *Globin Reduction Protocol: A Method for Processing Whole Blood RNA Samples for Improved Array Results*
- *GeneChip® Arrays Provide Optimal Sensitivity and Specificity for Microarray Expression Analysis*

Ordering Information

Complete GeneChip® Expression 3'– Amplification Reagents

	Contains	Quantity
900493 – One-Cycle Target Labeling and Control Reagents (30 rxns)	IVT Labeling Kit	1
	One-Cycle cDNA Synthesis Kit	1
	Sample Cleanup Module	1
	Poly-A RNA Control Kit	1
	Hybridization Controls	1
900494 – Two-Cycle Target Labeling and Control Reagents (30 rxns)	IVT Labeling Kit	1
	Two-Cycle cDNA Synthesis Kit	1
	Sample Cleanup Module	2
	Poly-A RNA Control Kit	1
	Hybridization Controls	1

The following kits can be ordered separately

		Product Name	Size	Part No.
Eukaryotic Reagents	Labeling Reagents	One-Cycle cDNA Synthesis Kit	30 rxns	900431
		Two-Cycle cDNA Synthesis Kit	30 rxns	900432
		T7-Oligo(dT) Promoter Primer Kit (already included in One- and Two-Cycle cDNA Synthesis Kits)	150 rxns	900375
		Sample Cleanup Module	30 rxns	900371
		IVT labeling Kit	30 rxns	900449
	Control Reagents	Eukaryotic Poly-A RNA Control Kit	~ 100 rxns	900433
		Hybridization Controls	30 rxns and 150 rxns	900454 900457
	Control Oligo B2 (already included in Hybridization Control Kit)	30 rxns	900301	
Prokaryotic Reagents	Control Reagents	Control Oligo B2	30 rxns	900301



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