

GeneChip® HT 3' IVT PLUS Reagent Kit (HT 3' IVT PLUS Kit)

An automated target preparation solution for expression analysis on Affymetrix' 3' IVT arrays

Introduction

Transcriptome profiling using Affymetrix' 3' IVT arrays is a well-proven technique for expression research and biomarker discovery. However, as the number of samples per study increases, the need arises for a target preparation process that continues to generate excellent results while remaining fast and efficient. The use of GeneChip® HT 3' IVT PLUS Reagent Kit (HT 3' IVT PLUS Kit) in combination with the Beckman Coulter® Biomek® FX^p Target Prep Express (TPE) System enables automation of the target preparation process, which significantly reduces the manual workload of the protocol. Using the automated protocol, researchers can process up to 96 samples in a single run with minimal hands-on time. Furthermore, the automated target preparation protocol retains the high sensitivity and specificity of the manual protocol, is optimized to work with total RNA from a wide range of samples, and does not require upfront removal of ribosomal RNA (rRNA).

HT 3' IVT PLUS Kit chemistry generates amplified and biotinylated complementary RNA (cRNA, also known as amplified RNA or aRNA) from poly-A RNA in a total RNA sample. HT 3' IVT PLUS

Kit uses reverse transcription by priming at the poly-A tail junction of RNA to provide gene expression profiles from mRNA. RNA amplification is based upon linear amplification and employs well-standardized T7 *in vitro* transcription (IVT) technology.

Features and benefits:

- Automated protocol enables the processing of up to 96 samples with minimal hands-on time
- Low RNA input requirement – as little as 50 ng total RNA input
- Protocol optimization with no initial primer annealing or cDNA purification step for a streamlined workflow
- Magnetic bead purification for enhanced recovery to generate sufficient target
- Built-in controls to provide effective troubleshooting

Specifications

cRNA yield	≥20 µg
% median CV	≤15%
Actin 3'/5' ratio	≤5.0
GAPDH 3'/5' ratio	≤2.0
Signal correlation Pearson r	≥0.95
Poly-A 3' detection	Present
Sample type	Total RNA from tissue, cell line, and whole blood*
Minimum sample input	50 ng
Sample input range	50 ng–500 ng

*If total RNA from whole blood is going to be used, the samples should be processed for globin reduction prior to target preparation with HT 3' IVT PLUS Kit.

Figure 1: The following signal correlation and fold-change correlation plots with superb Pearson r values between Affymetrix and three independent sites, demonstrating a performance consistency. At each site, replicates of hybridization targets were prepared from 50 ng minimum input MAQCA and MAQCB total RNA using HT 3' IVT PLUS Reagent Kit and were hybridized to Affymetrix® Human Genome U219 Arrays.

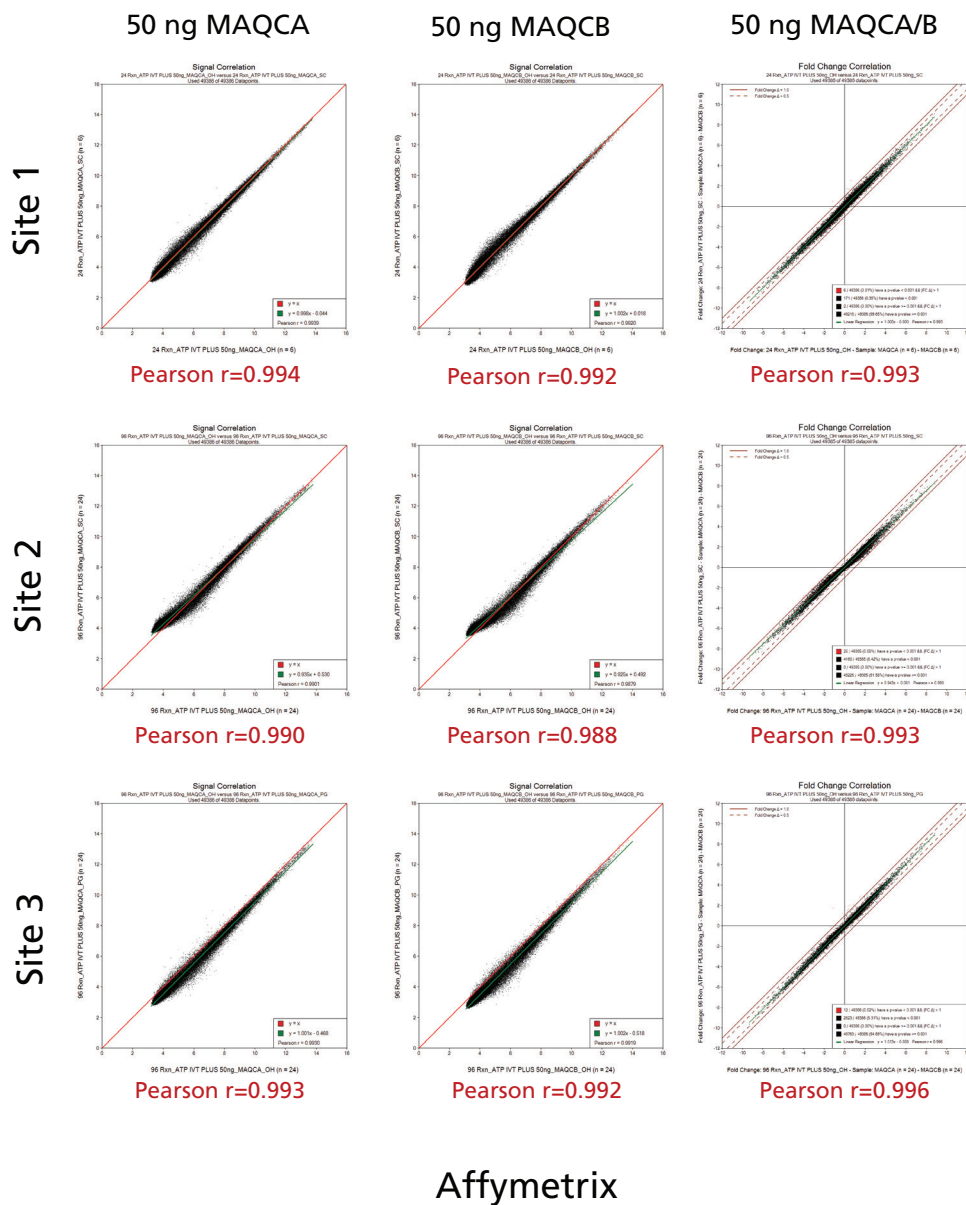


Table 2: The following coefficient of variation table demonstrates HT 3' IVT PLUS Kit's consistent assay performance in the customers' hands. Hybridization targets were prepared from 50 ng MAQCA and MAQCB total RNA by four sites (Affymetrix, Site 1, Site 2, and Site 3). Technical replicates were hybridized to Affymetrix® Human Genome U219 Arrays. Median coefficient of variation was calculated using all probe sets.

Site	RNA sample	Gene-level median coefficient of variation
Affymetrix	MAQCA	7.46%
Affymetrix	MAQCB	8.21%
Site 1	MAQCA	8.16%
Site 1	MAQCB	8.65%
Site 2	MAQCA	11.25%
Site 2	MAQCB	11.29%
Site 3	MAQCA	9.55%
Site 3	MAQCB	9.26%

Specification	15%
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Ordering information

Part number	Description	Details
902416	GeneChip® HT 3' IVT PLUS Reagent Kit	24 reactions
902417	GeneChip® HT 3' IVT PLUS Reagent Kit	96 reactions

Related products

Part number	Description	Details
900720	GeneTitan® Hybridization, Wash, and Stain Kit	Sufficient for 30 reactions
901530	GeneTitan® Hybridization, Wash, and Stain Kit for 3' IVT Arrays	Sufficient for 96 reactions
902519	Affymetrix® HT 3' IVT PLUS Consumables for Biomek® FX® Target Prep Express	Sufficient for 4x24 or 4x96 reactions

Recommended 3' IVT cartridge arrays

Part number	Description	Details
900470	GeneChip® Human Genome U133 Plus 2.0 Array	Contains 2 arrays
900466	GeneChip® Human Genome U133 Plus 2.0 Array	Contains 6 arrays
900467	GeneChip® Human Genome U133 Plus 2.0 Array	Contains 30 arrays
901837	GeneChipv PrimeView™ Human Gene Expression Array	Contains 10 arrays
901838	GeneChip® PrimeView™ Human Gene Expression Array	Contains 30 arrays
900495	GeneChip® Mouse Genome 430 2.0 Array	Contains 2 arrays
900496	GeneChip® Mouse Genome 430 2.0 Array	Contains 6 arrays
900497	GeneChip® Mouse Genome 430 2.0 Array	Contains 30 arrays
900505	GeneChip® Rat Genome 230 2.0 Array	Contains 2 arrays
900506	GeneChip® Rat Genome 230 2.0 Array	Contains 6 arrays
900507	GeneChip® Rat Genome 230 2.0 Array	Contains 30 arrays

Recommended 3' IVT array plates

Part number	Description	Details
901433	GeneChip® HT HG-U133+ PM 16-Array Plate	Contains 16 arrays
901261	GeneChip® HT HG-U133+ PM 24-Array Plate	Contains 24 arrays
901262	GeneChip® HT HG-U133+ PM 96-Array Plate	Contains 96 arrays
901605	Human Genome U219 16-Array Plate and Trays	Contains 16 arrays
901604	Human Genome U219 24-Array Plate and Trays	Contains 24 arrays
901595	Human Genome U219 96-Array Plate and Trays	Contains 96 arrays
901434	GeneChip® HT MG-430 PM 16-Array Plate	Contains 16 arrays
901257	GeneChip® HT MG-430 PM 24-Array Plate	Contains 24 arrays
901258	GeneChip® HT MG-430 PM 96-Array Plate	Contains 96 arrays
901435	GeneChip® HT RG-230 PM 16-Array Plate	Contains 16 arrays
901259	GeneChip® HT RG-230 PM 24-Array Plate	Contains 24 arrays
901260	GeneChip® HT RG-230 PM 96-Array Plate	Contains 96 arrays

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