

GeneChip® 3' IVT PLUS Reagent Kit

A complete sample preparation kit designed, developed, and optimized for expression analysis on Affymetrix' 3' Expression Arrays

Affymetrix' latest GeneChip® 3' IVT PLUS Reagent Kit (3' IVT PLUS Kit) enables the preparation of RNA samples for gene expression profiling with GeneChip® 3' Expression Arrays. This kit consists of all the reagents and a simple-to-use protocol required for preparing hybridization-ready targets from 50 to 500 ng of total RNA.

The 3' IVT PLUS assay demonstrates high sensitivity and specificity, is optimized to work with total RNA from a wide-range of samples including fresh-frozen tissues, cells, and cell lines, and does not require upfront removal of ribosomal RNA (rRNA). Total RNA derived from whole-blood samples is also compatible with 3' IVT PLUS Kit, so translational and clinical researchers can rapidly generate expression signatures from commonly used clinical sample types to develop non-invasive biomarker-directed tests.

The assay is a very streamlined and accurate and offers the following benefits:

- Low RNA input requirement – as little as 50 ng total RNA input for a single round of amplification to leave more samples for further studies

- Assay performance highly concordant with an established 3' expression assay kit, GeneChip® 3' IVT Express Kit (3' IVT Express Kit), for continuation of high-quality data generation without compromising the legacy data
- Protocol optimization with no initial primer annealing or cDNA purification step for a streamlined workflow
- Single-day protocol with appropriate inputs of total RNA for rapid sample processing
- Magnetic bead purification for enhanced recovery to generate sufficient target
- Built-in controls to assist in effective trouble shooting

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. 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.

Specifications

Labeled cRNA yield	≥15 µg
% Median CV	≤10%
Actin 3'/5' ratio	≤5.0
GAPDH 3'/5' ratio	≤2.0
Signal correlation Pearson r compared to Control* (all probe sets)	≥0.95
Poly-A 3' detection	Present
Sample type	Total RNA from tissue, cell line, whole blood** from eukaryotic organisms
Minimum sample input	50 ng
Sample input range	50 ng–500 ng
Validated array platforms	Cartridge, plate, and strip 3' expression arrays

*Control is the same sample prepared from 3' IVT Express Kit.

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

3' IVT PLUS Kit generates sufficient target from 50 ng total RNA using various tissues and cell lines with different sample quality.

The following bioanalyzer gel image for total RNA (Figure 1A) displays a range of degradation for the samples included in the assessment. The corresponding labeled cRNA yield (Figure 1B) captures the labeled cRNA yield generated by 3' IVT PLUS Kit for each of the samples. All samples generated sufficient hybridization target yield for 3' expression arrays such as GeneChip® Human Genome U133 Plus 2.0 Array.

Figure 1A: Bioanalyzer gel image of the total RNA from different fresh-frozen tissues and cell lines with the corresponding RIN values.

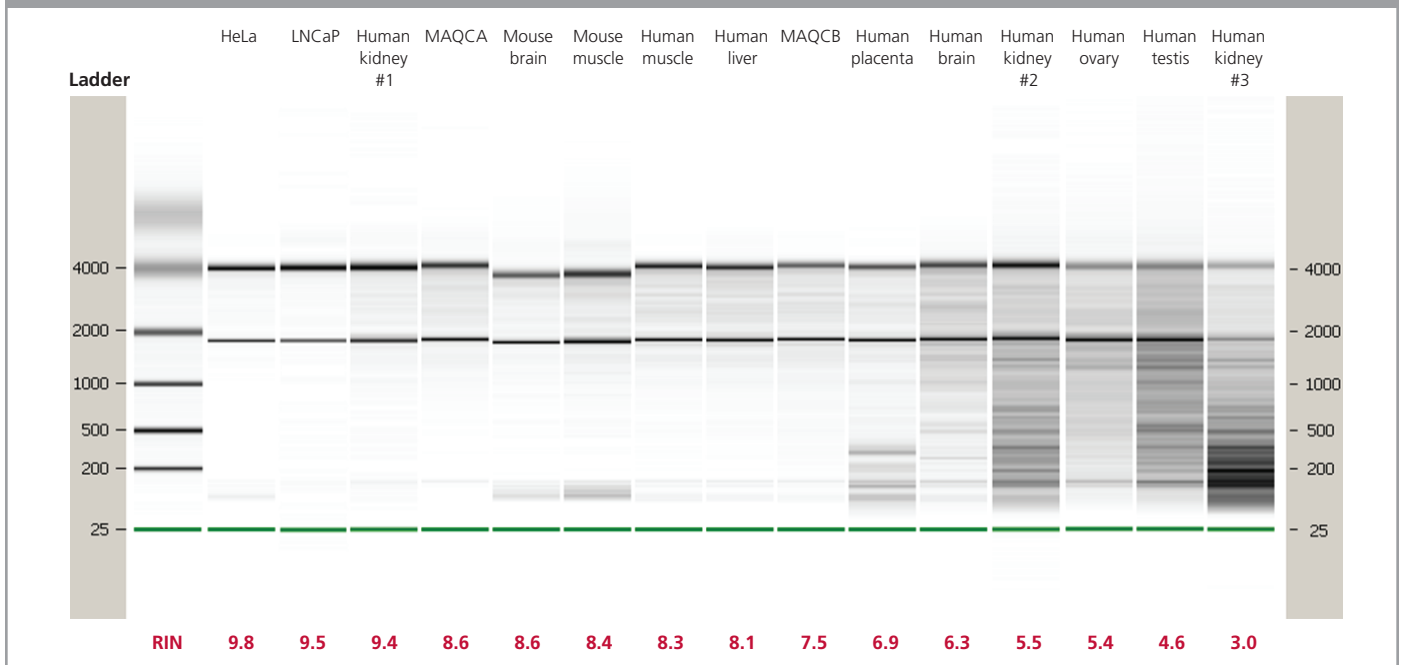
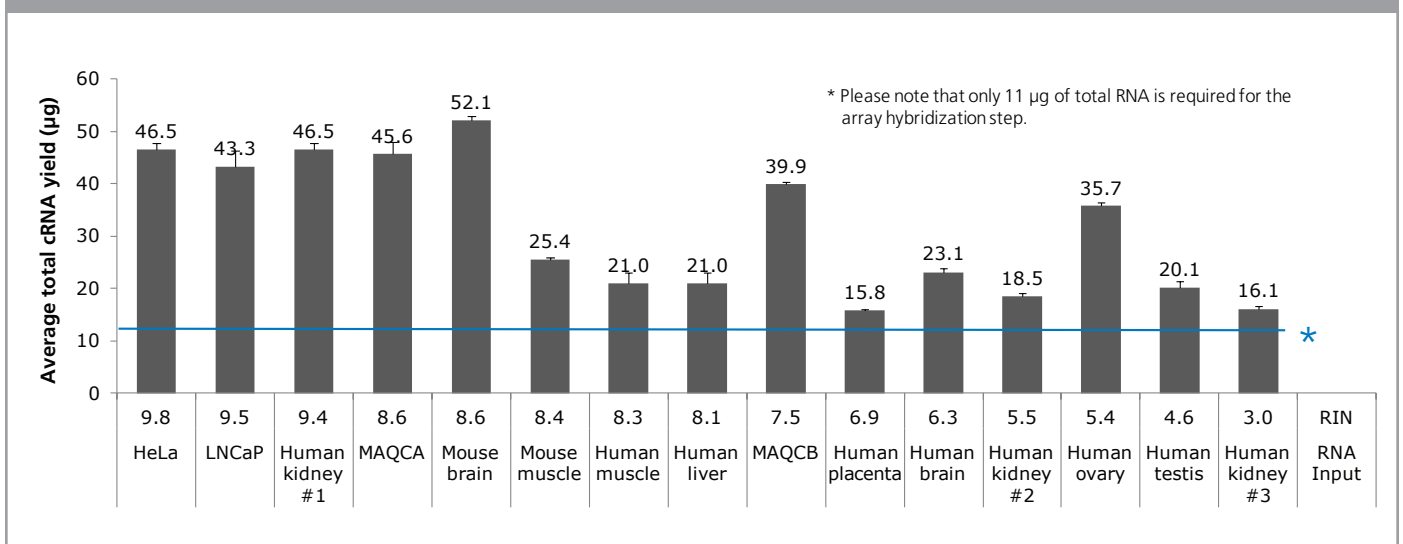
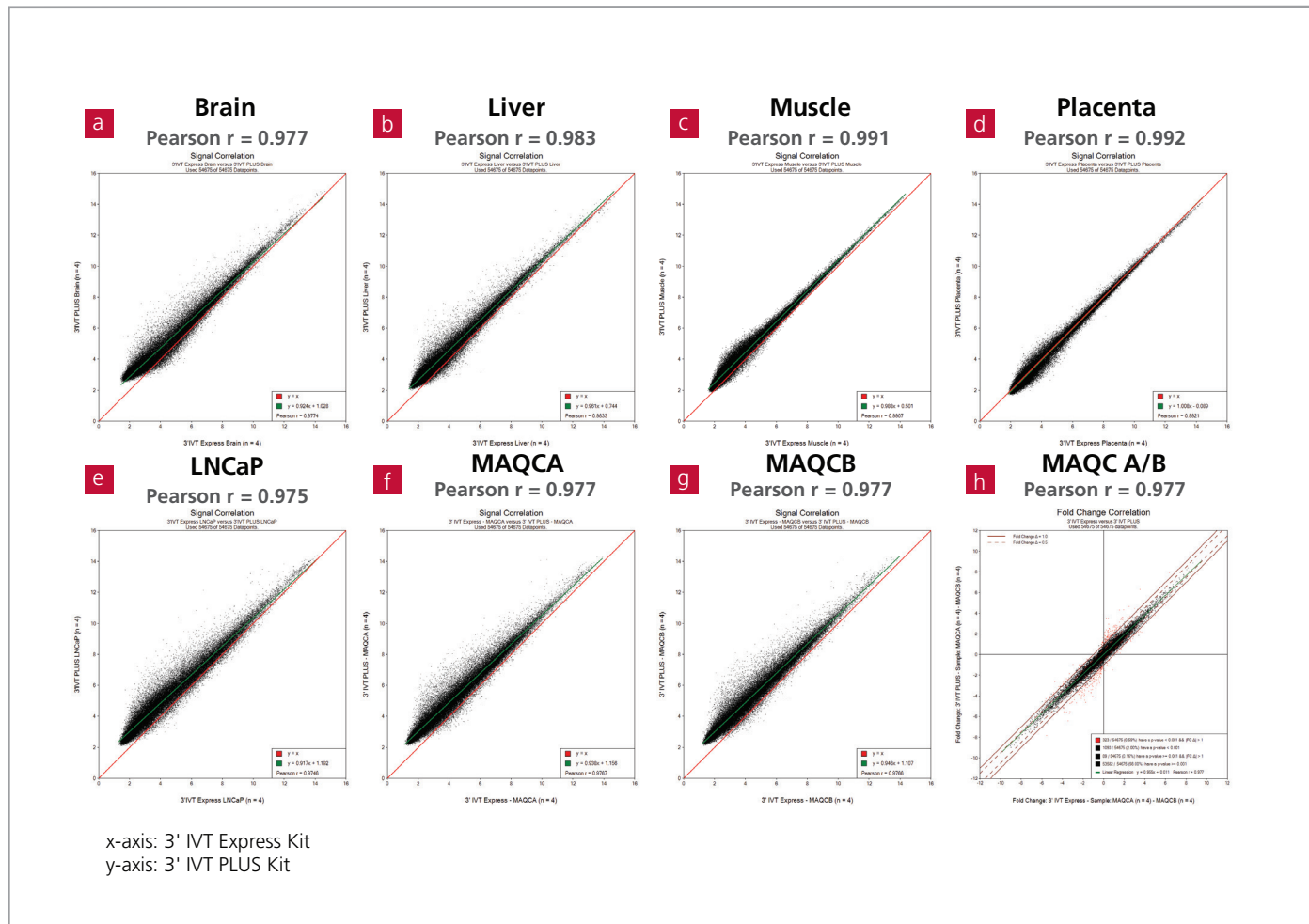


Figure 1B: The labeled cRNA yield generated by 3' IVT PLUS Kit for different fresh-frozen tissues and cell lines with the corresponding RIN values.



3' IVT PLUS Kit demonstrates high concordance with the widely used 3' IVT Express Kit.

The following correlation plots comparing 3' IVT PLUS Kit to 3' IVT Express Kit (Figure 2) demonstrate high Pearson r values indicating superb signal correlation (Figures a–g) and fold change correlation (Figure h) for various fresh-frozen tissues and cell lines. The target was generated using 50 ng input RNA material. 11 µg labeled cRNA was hybridized to GeneChip® Human Genome U133 Plus 2.0 Array for the concordance assessment.



Ordering information

Part number	Description	Details
902415	GeneChip® 3' IVT PLUS Reagent Kit	Sufficient for 10 reactions
902416	GeneChip® 3' IVT PLUS Reagent Kit	Sufficient for 30 reactions

Related reagents

Part number	Description	Details
900720	GeneChip® Hybridization, Wash, and Stain Kit	Sufficient for 30 reactions

Related arrays

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

*This table lists commonly used human, mouse, and rat 3' expression arrays. For model organisms arrays, please refer to www.affymetrix.com.

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P/N EMI03189 Rev. 2

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