MessageAmp™ Premier RNA Amplification Kit
MessageAmp™ III RNA Amplification Kit

Single-Round RNA Amplification for Affymetrix GeneChip® Analysis

- **Simplified Workflow**—New reagent formulations reduce the number of steps, resulting in an easy-to-use protocol
- **Most Efficient RNA Amplification Kit on the Market**—Start with as little as 20 ng of total RNA and produce enough biotin-labeled sample for GeneChip® analysis (RNA input range: 20–500 ng)
- **Fastest RNA Amplification/Labeling Available**—Prepare RNA samples (≥100 ng total RNA input) and hybridize to a GeneChip in a single day
- **Highly Consistent Results**—Less hands on time, less opportunity for errors. All reactions occur in a single tube or well

Introduction

DNA microarrays are the method of choice for assessing gene expression on a genome-wide scale. They can be used to evaluate the expression levels of thousands of genes in a single experiment. One of the challenges associated with microarray technology, however, is that large amounts of labeled antisense RNA (known as cRNA or aRNA) are required. T7 linear amplification, commonly referred to as the Eberwine method, overcomes this challenge by both increasing sample quantity and labeling the RNA. It is considered the gold standard for sample preparation in microarray-based expression profiling, and is the method recommended by Affymetrix, a leading manufacturer of microarrays, for its GeneChip® products. The new MessageAmp™ Premier and MessageAmp™ III Kits employ this proven methodology for enzymatic RNA amplification and labeling, but include innovations that both shorten the protocol and increase amplification efficiency.

Simplified Workflow

The MessageAmp Premier Kit consists of a set of optimized reagent and enzyme mixes for each major step of the RNA amplification process: reverse transcription, second strand cDNA synthesis, and in vitro transcription (IVT). By combining reagents into master mix formulations, the overall number of kit components and pipetting steps has been greatly reduced. Furthermore, the procedure has been streamlined so that the initial primer annealing step and the double-stranded cDNA purification step

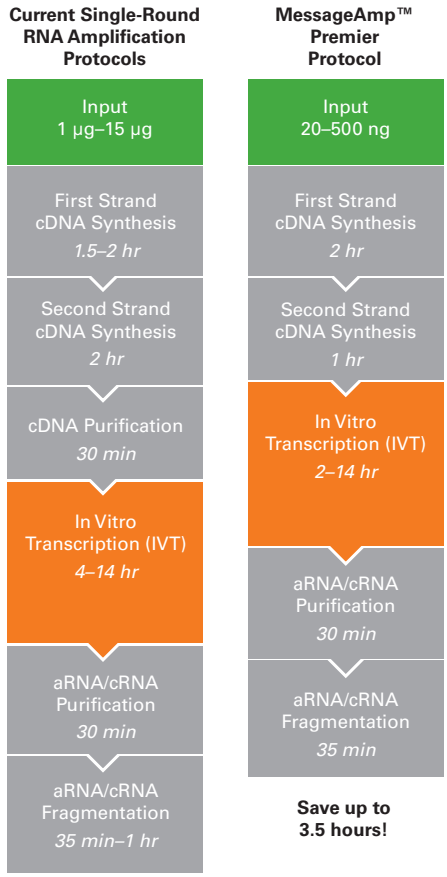

Figure 1. With the streamlined MessageAmp™ Premier protocol you can prepare samples for microarray analysis in a single day. The new kit employs optimized master mix formulations for improved workflow. Only 6 master mixes are required for all of the enzymatic reactions. The magnetic bead purification at the end of the procedure is both more efficient and requires less sample handling. The total time savings with MessageAmp Premier Kit is up to 3.5 hours (depending on amount of input RNA).

RNA Amount	IVT Incubation Time
20–50 ng	14 hr
50–100 ng	8 hr
100–200 ng	4–8 hr
200–500 ng	2–8 hr

Figure 2. Recommended IVT Incubation Times for the MessageAmp™ Premier Kit.

are no longer necessary (Figure 1). All reactions now take place in a single tube or in a single well of a 96-well plate, with sample transfer only for the purification of biotin-labeled product at the end of the procedure. By recommending that all reactions be incubated in a thermal cycler, another potential source of variability has also been removed. This new simplified workflow significantly reduces hands-on time, but more importantly, it improves amplification efficiency.

Highest Amplification Efficiency

Exhaustive optimization of each reaction step using the MessageAmp™ Premier Kit means that as little as 20 ng of total RNA can produce enough aRNA/cRNA for GeneChip® analysis (with a 14 hr IVT reaction). With higher input RNA levels, the recommended IVT incubation time decreases (Figure 2). This breakthrough in amplification efficiency opens the door to gene expression studies from RNA samples that previously were too limited for microarray analysis. For researchers with larger RNA samples, 100–500 ng, the dramatically reduced IVT incubation times facilitate higher throughput and shorter work days!

The experiment shown in Figure 3 compared yield and size of amplification products synthesized with a competitor's one cycle RNA amplification/labeling kit or with the new MessageAmp Premier Kit. Even though their reaction used 1 µg of input RNA vs. 100 ng for MessageAmp Premier, the size and yield of product was comparable. The aRNA yield from the MessageAmp Premier Kit is sufficient for one or more GeneChip hybridizations, even with much lower sample input (e.g., 20 ng of total RNA).

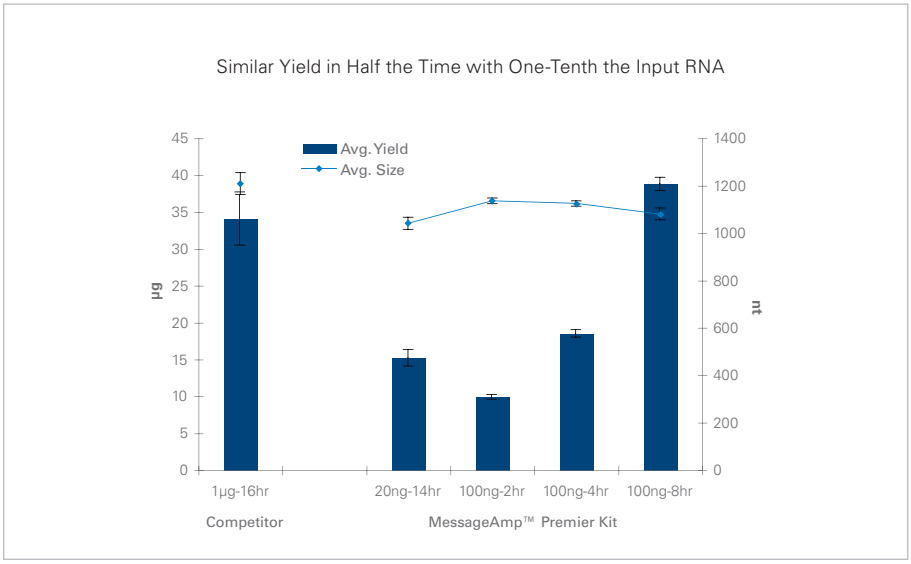

Figure 3. The amount and size of labeled aRNA/cRNA synthesized from HeLa RNA using either the MessageAmp™ Premier Kit or a competitor's one cycle amplification/labeling kit were compared. The minimum recommended amount of input RNA, 1 µg, was used in the competitor's kit with the recommended IVT incubation time of 16 hr. For the MessageAmp Premier Kit, both the minimum recommended amount of RNA, 20 ng and 100 ng of input RNA were amplified using the indicated IVT reaction times. aRNA/cRNA product size was comparable among the samples, but the amplification efficiency with the MessageAmp Premier Kit was much higher than with the competitor's kit. 100 ng of input RNA processed with the MessageAmp Premier Kit had similar yield to 10-fold the input RNA (1 µg) processed with the competitor kit.

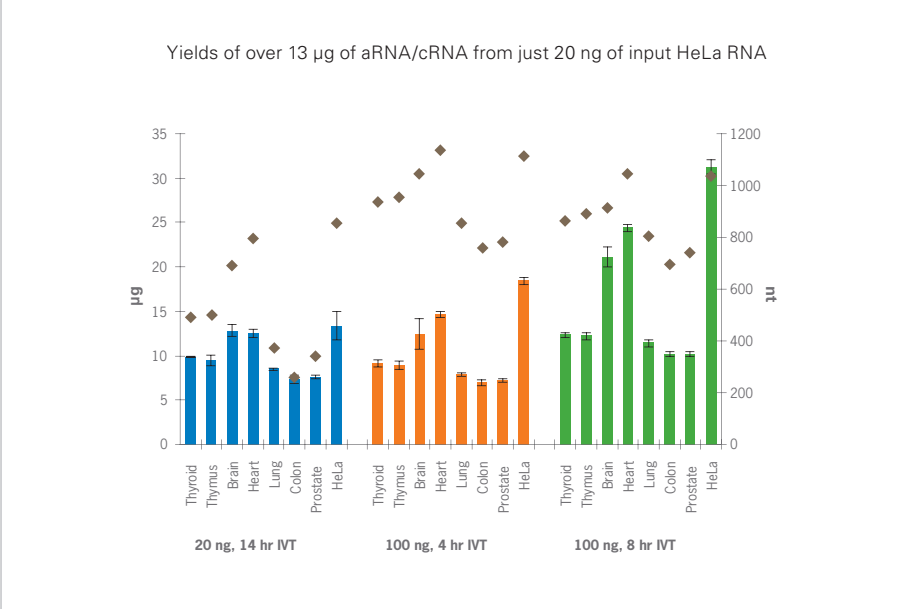

Figure 4. Triplicate RNA samples (20 or 100 ng) from HeLa cells or 7 tissues (Ambion FirstChoice® Total RNA) were amplified and labeled with the MessageAmp™ Premier Kit using the indicated IVT reaction times. Shown are the average amplified RNA yields (bars) and median size (diamonds).

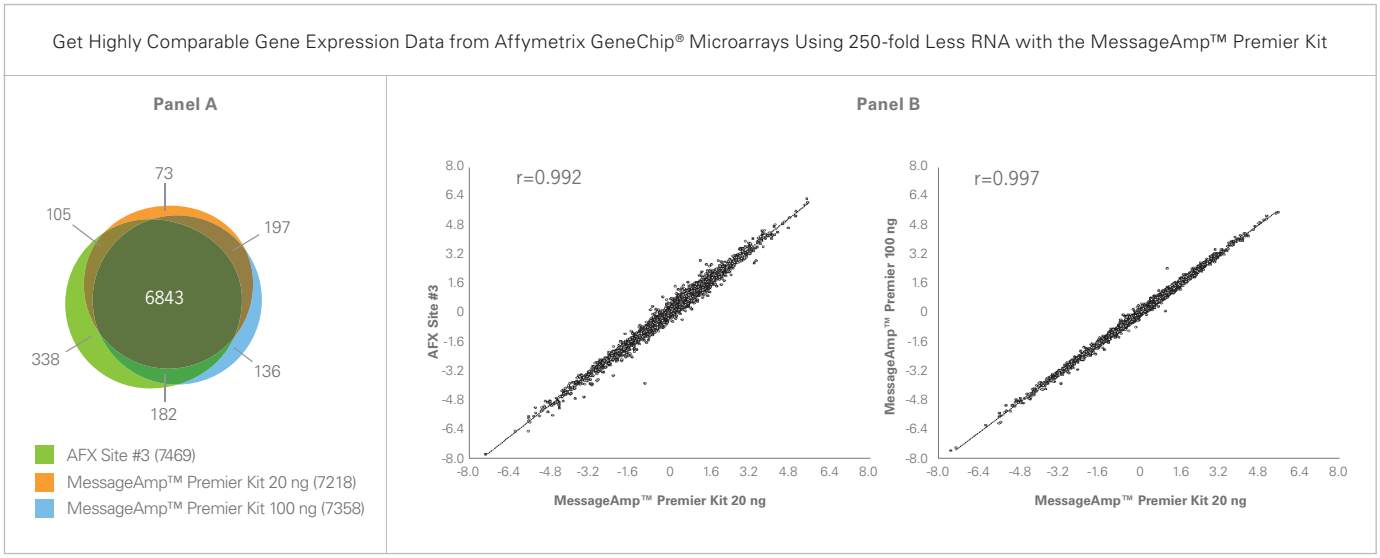

Figure 5. Panel A: Venn diagram representing present call concordance between samples prepared at MAQC AFX Site #3 (5000 ng input, 16 hr IVT) and samples prepared using the MessageAmp™ Premier Kit (20 ng input RNA, 14 hr IVT, and 100 ng input, 8 hr IVT). This illustrates the relationships between the Affymetrix GeneChip® probe sets that are considered "generally present" (as defined by the MAQC consortium) for sample A (Stratagene Universal Human Reference RNA), and sample B (Ambion Human Brain Reference RNA). **Panel B:** Scatter plots comparing Log_2 signal ratios from samples A and B ($log_{2(A/B)}$) using MAQC AFX site #3 5000 ng input data and MessageAmp Premier Kit 20 ng of input or 100 ng of input. Following the guidelines and comparison procedures recommended by the MAQC, only probe sets considered "generally present" in samples A and B are used for this analysis.

Furthermore, the average size of amplified RNA is consistently above 1,000 bp. This clearly illustrates the improvement in RNA amplification efficiency provided by the MessageAmp™ Premier Kit.

Fastest RNA Labeling/Amplification

Save a day: start with as little as 100 ng of total RNA and begin GeneChip® hybridization the same day (with a 4 hr IVT). Start with just 200 ng of total RNA, incubate the IVT for 2 hr, and be on a GeneChip in 5.5–6 hr!

Figure 4 shows the yield and size of MessageAmp Premier Kit amplification products from various amounts of RNA from several different human tissues. In some cases, 20 ng of input RNA provided enough labeled sample for GeneChip analysis. Increasing the amount of input RNA to 100 ng, however, provided enough labeled sample for GeneChip analysis even from tissues, such as thyroid and prostate, that are known to contain very little messenger RNA.

Comparable Microarray Data Using Only a Fraction of Total RNA

Figure 5 shows microarray data from The Microarray Quality Control Consortium (MAQC) that compares microarray results from samples prepared with either the MessageAmp Premier Kit (20 ng of input RNA, 14 hr IVT, and 100 ng of input, 8 hr IVT) or the well- established Affymetrix recommended kit and method (5000 ng of input, 16 hr IVT: AFX Site #3). The Venn diagram shows that concordance with MAQC AFX Site #3 is high for samples prepared using the MessageAmp Premier Kit from both 100 ng input RNA (95.5%) and 20 ng input RNA (96.3%). This high level of concordance is also seen with the MAQC AFX Sites #1 and #2 (data not shown). The scatter plots illustrate that signal ratios from both total RNA inputs (100 ng and 20 ng), amplified with the MessageAmp Premier Kit, show high correlation with the MAQC data (r>0.992). Additionally, signal ratios are very similar when comparing the two input levels (r>0.997). This high degree of correlation ensures that low input samples amplified and labeled for gene expression profiling using the MessageAmp Premier Kit will produce data quality comparable to methods previously reported using much higher total RNA inputs.

MessageAmp™ III RNA Amplification Kit

The MessageAmp III Kit offers a more efficient workflow and a more efficient reaction than previous generations of MessageAmp Kits. Its overall performance and workflow are similar to those of the MessageAmp Premier Kit; however, the minimal total RNA sample input for the MessageAmp III Kit is 35 ng, and it utilizes traditional glass fiber filter for cRNA purification. This results in cRNA recovery efficiency that is 15%–25% less than that of MessageAmp Premier Kit.

Magnetic Stand

The MessageAmp™ Premier Kit was developed using the Ambion Magnetic Stand-96, Cat #AM10027, for purification of reaction products.

For consistent results, we strongly recommend it for use with the MessageAmp Premier Kit.

Magnetic Bead-Based Purification vs. Glass Fiber Filter

The new MessageAmp™ Premier Kit utilizes magnetic bead-based purification of the aRNA/cRNA. Magnetic bead purification provides the following advantages over traditional glass fiber filter purification:

- More consistent results
- Higher yield
- No filter clogging
- Easier processing and less sample handling
- Higher throughput, making it ideal for automation

MessageAmp™ Premier and MessageAmp™ III Kits: The New Standards for GeneChip® Sample Prep/Labeling

Both the MessageAmp Premier Kit and MessageAmp III Kit incorporate extensive improvement to simplify the workflow and increase amplification efficiency. The kits include a series of master mixes that reduce the number of tubes, pipetting steps and steps in the actual procedure. The initial primer annealing step now takes place in the first strand cDNA synthesis reaction. The cDNA purification step has been eliminated from the workflow. All reactions, including first strand synthesis to the IVT reaction, take place in a single tube. This simplified workflow significantly shortens hands-on time and reduces operator-dependent errors. As with all MessageAmp™ kits, each lot of kits has passed rigorous QC, which includes Affymetrix GeneChip analysis and testing.

ORDERING INFORMATION

Description	Reactions	Part Number
MessageAmp™ Premier RNA Amplification Kit	10	4385821
	30	AM1792
	100	4383452
Magnetic Stand-96	1 each	AM10027
MessageAmp™ III RNA Amplification Kit	10	4383451
	30	AM1793

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