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TargetAmp™ 1-Round aRNA Amplification Kit 103

Cat. No. TAU1R5110 – 10 Reactions

Cat. No. TAU1R5124 – 24 Reactions

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1. Introduction

Features and Benefits of the TargetAmp 1-Round aRNA Amplification Kit 103:

1) Greater than 5,000-fold amplification of the Poly(A) RNA contained in a total RNA sample.
2) High yields of aRNA (cRNA) from as little as 25 ng of total RNA.
3) Utilizes an improved "Eberwine" linear RNA amplification process.
4) Virtually eliminates template-independent reactions.
5) Reproducible amplification results.
6) Single tube reaction.
7) No need to purify the cDNA transcription template prior to the *in vitro* transcription reaction.
8) Fast reaction times…1-round of amplification can be completed in about 6 hours.
9) Easy to use…color-coded tubes and a simplified pipetting scheme reduce labor and the possibility of error.

The TargetAmp™ 1-Round aRNA Amplification Kit 103 utilizes an improved "Eberwine" procedure[1] for amplifying Poly(A) RNA from as little as 25 ng of total cellular RNA. The kit has been developed for use with SuperScript III Reverse Transcriptase (Invitrogen; provided by the user). The complete TargetAmp 1-Round aRNA Amplification procedure can be completed in about 6 hours (Fig. 1).

1. **First-strand cDNA Synthesis:** The Poly(A) RNA component of a total RNA sample is reverse transcribed into first strand cDNA. The reaction is primed from a synthetic oligo(dT) primer containing a phage T7 RNA Polymerase promoter sequence at its 5′ end. First strand cDNA synthesis is catalyzed by SuperScript III Reverse Transcriptase (provided by the user) and performed at an elevated temperature to reduce RNA secondary structure.
2. **Second-strand cDNA Synthesis:** The RNA component of the cDNA:RNA hybrid produced in Step 1 is digested into small RNA fragments using an RNase H enzyme. The RNA fragments then prime second-strand cDNA synthesis. The resulting product is a double-stranded cDNA containing a T7 transcription promoter in an orientation that will generate anti-sense RNA (aRNA; also called cRNA) during the subsequent *in vitro* transcription reaction. The cDNA produced can be used in the *in vitro* transcription reaction without the need for purification.
3. ***In Vitro* Transcription of aRNA:** High yields of aRNA (cRNA) are produced in a rapid *in vitro* transcription reaction that utilizes the double-stranded cDNA produced in Step 2.

Performance Specifications and Quality Control

The TargetAmp 1-Round aRNA Amplification Kit 103 is function-tested in a control reaction. The kit must produce at least 20 µg of aRNA (cRNA) from 200 ng of HeLa Total RNA Control, corresponding to a greater than 5,000-fold amplification of the Poly(A) RNA assuming that 2% of the HeLa Total RNA Control is Poly(A) RNA. A negative control reaction ("no-RNA" control) produces less than 1 µg of aRNA.

2. Kit Contents

The kit components are supplied in tubes with colored caps for easier identification.

The kit has been developed for use with and will provide optimal results with SuperScript® III Reverse Transcriptase (Invitrogen Corp.; provided by the user).

Component Name	Tube Label	Volume		Cap Color
		10-rxn	24-rxn	
TargetAmp T7-Oligo(dT) Primer A	T7-Oligo(dT) Primer A	15 µl	30 µl	Red
TargetAmp Reverse Transcription PreMix-SS	RT PreMix-SS	25 µl	50 µl	
TargetAmp DNA Polymerase PreMix-SS 1	DNA Polymerase PreMix-SS 1	60 µl	125 µl	
TargetAmp DNA Polymerase-SS 1	DNA Polymerase-SS 1	10 µl	18 µl	
TargetAmp T7 RNA Polymerase	T7 RNA Polymerase	50 µl	110 µl	Green
TargetAmp T7 Transcription Buffer	T7 Transcription Buffer	50 µl	110 µl	
100 mM ATP	ATP	50 µl	100 µl	
100 mM CTP	CTP	50 µl	100 µl	
100 mM GTP	GTP	50 µl	100 µl	
100 mM UTP	UTP	50 µl	100 µl	
RNase-Free DNase I	DNase I	30 µl	55 µl	
Dithiothreitol (DTT)	DTT	60 µl	125 µl	Clear
HeLa Total RNA Control (40 ng/µl)	HeLa Total RNA Control	10 µl	10 µl	
RNase-Free Water	RNase-Free Water	500 µl	500 µl	

Storage: Upon receipt of this kit, remove the tube containing the HeLa Total RNA Control and store it at –70°C to –80°C. Store the remainder of the kit at –20°C.

Additional Required Reagents and Equipment:
SuperScript® III Reverse Transcriptase (Invitrogen Corp.)
Thermocycler or water bath
Microcentrifuge
RNase-Free Water
RNeasy® MinElute® Cleanup Kit
or
RNeasy Mini Kit (Qiagen) (see "aRNA Purification" page 12 for details)

3. Preparation

Assessing the Quality of the Total RNA:

The success of microarray experiments is strongly influenced by the quality of the RNA. RNA quality has two components…purity of the RNA (or absence of contaminants) and integrity (intactness) of the RNA. RNA quality should be assessed prior to every RNA amplification reaction. Poor quality RNA is the most common cause of sub-optimal RNA amplification results!

RNA Purification Methods and RNA Purity. Total cellular RNA, isolated by a number of methods, can be amplified successfully using the TargetAmp 1-Round aRNA Amplification Kit 103. However, it is very important that the purified RNA be free of salts, metal ions, ethanol and phenol which can inhibit the enzymatic reactions performed in the RNA amplification process. Commonly used RNA extraction and purification methods that are compatible with the TargetAmp RNA amplification process include but are not limited to:

TRIzol®/TRI Reagent®, a homogeneous solution of the powerful denaturants guanidinium isothiocyanate and phenol, is very effective at extracting the RNA from the cells. However all traces of guanidinium salts and phenol must be removed from the RNA sample prior to the RNA amplification process. If you precipitate the RNA from TRIzol-extracted cells, be sure to wash the RNA pellet at least two times with cold 70-75% ethanol to remove all traces of phenol and guanidinium salts. Air dry the RNA pellet (do not use a vacuum centrifuge) to remove residual ethanol. Then, resuspend the RNA in RNase-Free water. If you purify the RNA from TRIzol-extracted cells by column purification methods, please read the section "Spin Columns" immediately following.

Spin Columns (e.g., the RNeasy MinElute Cleanup Kit and RNeasy Mini Kit from Qiagen) are effective in purifying RNA samples that are free of the contaminants that may inhibit the RNA amplification process. Spin columns can be used with most RNA extraction procedures (e.g. TRIzol reagent). If using spin columns, follow the manufacturer's instructions closely, especially if an ethanol wash of the RNA is performed prior to the RNA elution step. Then, elute the RNA from the column membrane using RNase-Free water.

Salt-Fractionation: RNA purification that employs gentle salt-fractionation, such as Epicentre's ArrayPure™ Nano-scale RNA Purification Kit, routinely produce the highest yield of intact RNA without the use of phenol, guanidinium salts, or columns. The ArrayPure kit has been developed for total RNA purification from 1-10,000 cells obtained by laser-capture methods such as Laser Capture Microdissection (LCM), from biopsy samples, from cell culture or quick-frozen tissue. To purify RNA from >10,000 cells, Epicentre's MasterPure™ RNA Purification Kit is recommended. When using these kits, be sure to wash the RNA pellet at least two times with cold 70-75% ethanol to remove all traces of salts. Air dry the RNA pellet (do not use a vacuum centrifuge) to remove residual ethanol. Then, resuspend the RNA in RNase-Free water. The ArrayPure kit and MasterPure kit are not recommended for purification of RNA from tissue samples preserved with RNA*later*® or RNA*later*-ICE.

RNA Integrity. Successful microarray analysis using amplified RNA is dependent on an RNA sample that contains full-length, intact Poly(A) RNA. The most commonly used

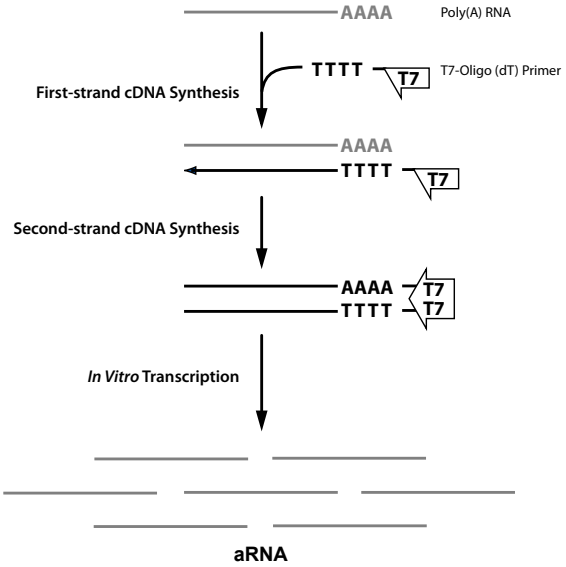

Figure 1. TargetAmp™ 1-Round aRNA Amplification Kit 103 Procedure.

methods for assaying RNA integrity are by denaturing agarose gel electrophoresis or using an Agilent 2100 Bioanalyzer.

The advantages of denaturing agarose gel electrophoresis are its low cost and ready availability of the reagents required. Denaturing gel electrophoresis separates the RNAs by size (electrophoretic mobility) under denaturing conditions. Denaturing conditions are necessary to eliminate inter- and intra-molecular secondary structure within the RNA sample which may cause degraded RNA to appear intact. Following electrophoresis, the denaturing gel is stained with, for example, ethidium bromide and the user looks for the highly stained 18S and 28S rRNAs. These bands should be sharp and discrete with an absence of smearing under either. Based on these visual observations, the user infers that the mRNA in the sample is equally intact (or degraded). If the rRNA bands appear degraded, as evidenced by smearing under each, the RNA sample should be discarded and a new sample of total RNA purified. Ideally, the ethidium bromide stained 28S rRNA band should appear to be about twice as intense as the 18S rRNA band. The main disadvantage of denaturing gels is that they require a minimum of 1 µg of total RNA be loaded per lane…a quantity of RNA that often is not expendable for those performing RNA amplification.

The Agilent 2100 Bioanalyzer is currently the preferred method for evaluating the integrity of an RNA sample. Like a denaturing gel, the bioanalyzer separates the RNAs by size (electrophoretic mobility). However, in contrast to a denaturing gel, the 2100 Bioanalyzer consumes as little as 5 ng of total RNA per well when using the manufacturer's RNA 6000 Nano LabChip®. When analyzing the RNA sample using the Agilent 2100 Bioanalyzer, the 18S and 28S rRNA species should appear as distinct, sharp peaks on the electropherogram. A slightly increased baseline, indicative of the 1-5% Poly(A) RNA contained in the sample, can be seen between the two peaks.

Maintaining an RNase-free Environment:

Ribonuclease contamination is a significant concern for those performing RNA amplification. The ubiquitous RNase A is a highly stable and active ribonuclease that can contaminate any lab environment and is present on human skin. All components of the TargetAmp 1-Round aRNA Amplification Kit 103 have been tested to ensure the lack of contaminating ribonuclease activities. However, creating an RNase-free work environment and maintaining RNase-free solutions is critical for performing successful RNA amplification reactions. Therefore, we strongly recommend that the user:

1) Autoclave all tubes and pipette tips that will be used in the RNA amplification reactions.
2) Always wear gloves when handling samples containing RNA. Change gloves frequently especially after touching potential sources of RNase contamination such as door knobs, pens, pencils, and human skin.
3) Always wear gloves when handling kit components. Do not pick up any kit component with an ungloved hand.
4) Keep all kit components tightly sealed when not in use. Keep all tubes containing RNA tightly sealed during the incubation steps.

Input Total RNA and aRNA Yield:

The TargetAmp 1-Round aRNA Amplification Kit 103 is extremely efficient at amplifying very small quantities of Poly(A) RNA in a total RNA preparation. The TargetAmp kit has been evaluated for production of aRNA from as little as 25 ng of total RNA using the HeLa Total RNA control provided in the kit. User results may vary depending on the quality of the total RNA sample and the Poly(A) content of the sample.

Table 1. Yields of aRNA (cRNA) obtained using the TargetAmp™ 1-Round aRNA Amplification Kit 103. Results are the average of multiple experiments using the HeLa Total RNA control provided in the kit.

HeLa Control total RNA	HeLa Poly(A) RNA [assume 2% of HeLa total RNA is Poly(A) RNA]	aRNA Yield	Fold-Amplification of HeLa Poly(A) RNA [aRNA yield / Poly(A) content]
25 ng	0.5 ng	3.1 µg (3100 ng)	6200
50 ng	1 ng	5.3 µg (5,300 ng)	5300
100 ng	2 ng	10.8 µg (10,800 ng)	5600
200 ng	4 ng	22.6 µg (22,600 ng)	5650
400 ng	8 ng	47.2 µg (47,200 ng)	5900
500 ng	10 ng	75.6 µg (75,600 ng)	7500

Important! *The TargetAmp 1-Round aRNA Amplification Kit 103 has been optimized for producing >5,000-fold amplification of the Poly(A) RNA from 25-500 ng of total cellular RNA per reaction. Amplifying >500 ng of total RNA in a single TargetAmp 1-Round aRNA Amplification Kit 103 reaction may result in less than 5,000-fold amplification of the Poly(A) RNA and may result in under-representation of some Poly(A) RNA sequences in the aRNA produced. Therefore, if it is desirable to perform an amplification reaction using >500 ng of total RNA, we strongly recommend that the user perform multiple reactions, each containing up to but not exceeding 500 ng of total RNA.*

aRNA Purification:

The appropriate aRNA purification process (Step D) to use is dependent on the expected yield of aRNA. Use the table on page 8 to estimate the yield of aRNA expected from the amount of total RNA used in each amplification reaction. Then:

1) If the expected yield of aRNA is <40 µg, purify the aRNA using the Qiagen MinElute Cleanup Kit.
2) If the expected yield of aRNA is >40 µg, purify the aRNA using the Qiagen RNeasy Mini Kit.

Additional Suggestions:

Familiarize Yourself with the TargetAmp Kit and Procedure:

The TargetAmp 1-Round aRNA Amplification Kit 103 includes many reagents. Before starting, please read this protocol carefully and familiarize yourself with each kit component and in which step of the RNA amplification process it is used. Be sure to wear gloves when handling the kit components.

Importance of Running the TargetAmp 1-Round aRNA Control Reaction:

We strongly recommend that those who are not experienced with the TargetAmp 1-Round aRNA Amplification Kit 103 perform a control amplification reaction (Appendix 1) prior to committing a precious sample. HeLa total RNA is provided with the kit as a control.

Performing the TargetAmp 1-Round aRNA Amplification Reactions:

We recommend that all reactions be performed in sterile 0.2-ml thin-walled tubes using sterile pipette tips and recently calibrated pipettors. Very small volumes of some kit components are required for each reaction. Therefore, we recommend the user prepare Master Mixes of reaction components when amplifying multiple samples.

Some Simple but Important Factors for Obtaining Optimal Results:

1) Familiarize yourself with the kit by running a control reaction (Appendix 1) before committing a precious sample.
2) Use up to but not more than 500 ng of total RNA per reaction.
3) Assemble the *in vitro* transcription reaction (Step C) at room temperature. Do not exceed the *in vitro* transcription reaction time indicated in the procedure.
4) Use the appropriate RNA purification columns for purifying the aRNA produced.
5) Optional stopping points are noted following the second-strand cDNA synthesis step (Part B) and after purification of the aRNA (Part D).

4. RNA Amplification Procedure

Please read through the TargetAmp 1-Round aRNA Amplification Kit 103 procedure carefully before beginning. We strongly recommend that those who are not experienced with the TargetAmp kit perform a control amplification reaction (Appendix 1) prior to committing a precious sample. A HeLa total RNA control is provided with the kit.

An abbreviated (single page) protocol is presented on page 14 for users experienced in using this kit.

A. First-strand cDNA Synthesis

SuperScript III Reverse Transcriptase (Invitrogen) is required for use in this Step. The SuperScript III enzyme is provided by the user. The total RNA sample must be free of contaminating salts, metal ions, ethanol, and phenol. For best results, the RNA sample should be dissolved in RNase-Free water.

Required in Step A

Component Name	Tube Label	Cap Color
TargetAmp T7-Oligo(dT) Primer A	T7-Oligo(dT) Primer A	Red
TargetAmp Reverse Transcription PreMix-SS	RT PreMix-SS	
Dithiothreitol	DTT	Colorless
RNase-Free Water	RNase-Free Water	

Incubation temperatures performed in Step A: 50°C and 65°C.

Important! *The TargetAmp 1-Round aRNA Amplification Kit 103 has been optimized for producing >5,000-fold amplification of the Poly(A) RNA from 25-500 ng of total cellular RNA per reaction. Amplifying >500 ng of total RNA in a single TargetAmp 1-Round aRNA Amplification Kit 103 reaction may result in less than 5,000-fold amplification of the Poly(A) RNA and may result in under-representation of some Poly(A) RNA sequences in the aRNA produced. Therefore, if it is desirable to perform an amplification reaction using >500 ng of total RNA, we strongly recommend that the user perform multiple reactions, each containing up to but not exceeding 500 ng of total RNA.*

1. Anneal the TargetAmp T7-Oligo(dT) Primer A to the RNA sample. If a "no template" control reaction is performed, substitute 2 µl of RNase-Free Water for the Total RNA sample.

x	µl	RNase-Free Water
x	µl	Total RNA sample (25-500 ng)
1	µl	TargetAmp T7-Oligo(dT) Primer A
3	µl	Total reaction volume

2. Incubate at 65°C for 5 minutes in a water bath or thermocycler.
3. Chill on ice for 1 minute. Centrifuge briefly in a microcentrifuge.
4. Prepare the First-strand cDNA Synthesis Master Mix.

   For each first-strand cDNA synthesis reaction, combine on ice:

1.5	µl	TargetAmp Reverse Transcription PreMix-SS
0.25	µl	DTT
0.25	µl	SuperScript III Reverse Transcriptase (200 U/µl)
2	µl	Total reaction volume

   Important! *Be sure to use SuperScript III Reverse Transcriptase. Do not use the SuperScript 5X Buffer or the DTT that is provided with the enzyme.*
5. Gently mix the First-strand cDNA Synthesis Master Mix and then add 2 µl of it to each reaction.
6. Gently mix the reactions and then incubate each at 50°C for 30 minutes in a water bath or thermocycler. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 50°C.

B. Second-strand cDNA Synthesis

Required in Step B

Component Name	Tube Label	Cap Color
TargetAmp DNA Polymerase PreMix-SS 1	DNA Polymerase PreMix-SS 1	Red
TargetAmp DNA Polymerase-SS 1	DNA Polymerase-SS 1	

Incubation temperatures performed in Step B: 65°C and 80°C.

1. Prepare the Second-strand cDNA Synthesis Master Mix.

   For each second-strand cDNA synthesis reaction, combine on ice:

4.5	µl	TargetAmp DNA Polymerase PreMix-SS 1
0.5	µl	TargetAmp DNA Polymerase-SS 1
5	µl	Total reaction volume

2. Gently mix the Second-strand cDNA Synthesis Master Mix and then add 5 µl of it to each reaction.
3. Gently mix the reactions and then incubate at 65°C for 10 minutes in a water bath or thermocycler. Centrifuge briefly in a microcentrifuge.

   Important! Be sure to incubate the reactions at 65°C.
4. Incubate the reactions at 80°C for 3 minutes.

   Centrifuge briefly in a microcentrifuge then chill on ice.

   Note: *If desired, the reactions can now be frozen and stored overnight at –20°C.*

C. *In Vitro* Transcription of aRNA

Required in Step C

Component Name	Tube Label	Cap Color
TargetAmp T7 RNA Polymerase	T7 RNA Polymerase	Green
TargetAmp T7 Transcription Buffer	T7 Transcription Buffer	
100 mM ATP	ATP	
100 mM CTP	CTP	
100 mM GTP	GTP	
100 mM UTP	UTP	
Dithiothreitol	DTT	Clear
RNase-Free Water	RNase-Free Water	
RNase-Free DNase I	DNase I	Green

Incubation temperatures performed in Step C: 37°C and 42°C.

1. Warm the TargetAmp T7 RNA Polymerase to room temperature. Thaw the remaining *in vitro* transcription reagents at room temperature. If a precipitate is visible in the thawed TargetAmp T7 Transcription Buffer, heat the Buffer to 37°C until it dissolves. Keep the TargetAmp T7 Transcription Buffer at room temperature.
2. Thoroughly mix the thawed TargetAmp T7 Transcription Buffer.

   Important! *If a precipitate is visible in the thawed TargetAmp T7 Transcription Buffer, heat the Buffer to 37°C until it dissolves. Mix the Buffer thoroughly. Keep the Buffer at room temperature.*
3. Prepare the *In Vitro* Transcription Master Mix.

   For each *in vitro* transcription reaction, combine at room temperature:

13.6	µl	RNase-Free Water
4	µl	TargetAmp T7 Transcription Buffer
3.6	µl	ATP
3.6	µl	CTP
3.6	µl	GTP
3.6	µl	UTP
4	µl	DTT
4	µl	TargetAmp T7 RNA Polymerase
40	µl	Total reaction volume

4. Gently mix the *In Vitro* Transcription Master Mix and then add 40 µl of it to each reaction.
5. Gently mix the reactions and then incubate at 42°C for 4 hours in a thermocycler. If the thermocycler has a heated lid function, heat the lid only if the temperature of the lid can be maintained at about 42°C. If the lid temperature can not be maintained at about 42°C, then perform the incubations without heating the lid.

Important! *Do not exceed 4 hour incubation at 42°C. Optimal yield and quality (length) of aRNA is achieved in 4 hours.*

6. Add 2 µl of RNase-Free DNase I to each reaction.
   Mix gently and then incubate each at 37°C for 15 minutes.

D. aRNA Purification

The purification column to use is dependent upon the expected yield of aRNA. Use the table on page 7 to estimate the yield of aRNA expected from the amount of total RNA used in each amplification reaction. Then,

If the expected yield of aRNA is <40 µg: purify the aRNA using the Qiagen RNeasy MinElute Cleanup Kit. (Qiagen cat. no. 74204)

If the expected yield of aRNA is >40 µg: purify the aRNA using the Qiagen RNeasy Mini Kit. (Qiagen cat. no. 74014)

Use the RNase-Free Water that is provided in the MinElute Cleanup Kit or the RNeasy Mini Kit. The following procedure should be used with either the MinElute Cleanup Kit or the RNeasy Mini Kit.

1. Prepare 350 µl of RLT/β-ME Solution for each sample. Combine the RLT/β-ME in the ratio of 1 ml of Buffer RLT (provided in the RNeasy kit) with 10 µl of β-ME (β-mercaptoethanol) as described in the RNeasy kit's handbook.
2. Prepare 650 µl of RPE Solution for each sample by diluting 1 volume of Buffer RPE (provided in the purification kits) with 4 volumes of 96-100% ethanol as described in the purification kit's handbook.
3. To each sample add:

48	µl	RNase-Free Water
350	µl	RLT/β-ME Solution
250	µl	100% Ethanol

4. Apply each sample to the purification kit's spin column in a 2 ml collection tube. Centrifuge at >8,000 x g for 15 seconds. Discard the flow-through.
5. Apply 650 µl RPE Solution onto the column.
   Centrifuge at >8,000 x g for 15 seconds. Discard the flow-through.
6. Apply 650 µl 80% ethanol onto the column.
   Centrifuge at >8,000 x g for 15 seconds. Discard the flow-through.
7. Transfer the spin column into a new collection tube.
   Centrifuge at full speed for 5 minutes.
8. Transfer the spin column to a 1.5 ml collection tube. Elute the aRNA:

   If using the MinElute Cleanup Column, apply 15 µl of RNase-Free Water directly onto the center of the silica-gel membrane. Wait for 5 minutes. Centrifuge at full speed for 1 minute.

   If using the RNeasy Mini Column, apply 25-50 µl of RNase-Free Water directly onto the center of the silica-gel membrane. Wait for 5 minutes. Centrifuge at full speed for 1 minute.

Note: *If desired, the reactions can now be quick frozen (e.g., dry ice/ethanol bath) and stored overnight at –80°C.*

E. Quantifying the Concentration, Yield and Fold-Amplification of the aRNA

Concentration and yield: The concentration of the aRNA can be readily determined using a NanoDrop® ND-1000 UV-Vis Spectrophotometer available from NanoDrop Technologies. Alternately, due to the high yield of aRNA that is produced by a TargetAmp reaction, the yield and concentration of aRNA can be determined by standard UV spectroscopy.

1. Prepare a dilution of the aRNA into the minimum volume of water or TE Buffer (10 mM Tris-HCl [pH 7.5], 1 mM EDTA) required by the spectrophotometer cuvette that will be used.
2. Zero the spectrophotometer at 260 nm using the diluents (water or TE buffer) that was used to dilute the aRNA sample.
3. Measure and record the absorbance of the diluted aRNA at 260 nm (A_{260}).
4. Calculate the concentration of the aRNA. Use the conversion factor that an A_{260} reading of 1.0 is equal to an RNA concentration of 40 µg/ml.
   aRNA concentration = (A_{260} reading) x (dilution factor) x (40 µg/ml).
   Example: Dilution for A_{260} measurement = 1:100 with an A_{260} of the 1:100 dilution = 0.15.
   aRNA concentration = (0.15) x (100) x (40 µg/ml) = 600 µg/ml = (0.6 µg/µl) aRNA.
5. Calculate the yield of aRNA using the formula:
   Yield of aRNA = (aRNA Concentration) x (Volume of aRNA).
   Example: 50 µl of aRNA recovered from column, 0.6 µg/µl aRNA determined in Part E, Step 4.
   aRNA yield = (0.6 µg/µl) x (50 µl) = 30 µg of aRNA.

   In this example, 1 µl of 0.6 µg/µl of aRNA was used for the spectrophotometer reading so there are now 29.4 µg of aRNA remaining.

   Fold amplification: The fold-amplification of the reaction can be calculated once the yield of the aRNA has been determined. However, if the input RNA was total RNA, an accurate calculation of fold-amplification requires knowledge of the Poly(A) content of the original total RNA sample. If the Poly(A) content of the sample is not known, a commonly used assumption is that Poly(A) RNA constitutes 2% of the RNA in a total RNA sample.

   Fold-amplification = (amount of aRNA produced) / ([amount of total RNA input] x [Percentage of Poly(A) RNA in the total RNA sample]).

   Example: Amount of input total RNA = 200 ng, Percentage of Poly(A) RNA in the sample (assumed) = 2% (0.02), Amount of aRNA produced = 30,000 ng (30 µg).

   Fold-amplification = 30,000 ng / (200 ng x 0.02) = 7,500.

F. Assessing the Size of the aRNA Produced

A TargetAmp 1-Round aRNA Amplification Kit 103 reaction typically produces aRNA with a size distribution between 200-3,000 bases with an average size of about 1,000 bases.

Sizing the aRNA by denaturing gel electrophoresis: The advantages of denaturing agarose gel electrophoresis are its relatively low cost and ready availability of the reagents required. When assessing the size distribution of the aRNA, load at least 1 µg into the well of a 1% formaldehyde-agarose gel. Load RNA size markers that cover the size range of approximately 100-2,000 bases.

Sizing the aRNA using the Agilent 2100 Bioanalyzer: Dilute an aliquot of the aRNA with water to approximately 100 ng/µl. Using the Agilent RNA 6000 Nano LabChip, load 1 µl of the diluted aRNA per well. It is advisable to load and run duplicates of each sample tested. If a control reaction containing no input RNA was performed, load 1 µl of this sample as well. Run the samples per instrument procedure.

Abbreviated RNA Amplification Procedure for users experienced in using this kit.

First-strand cDNA Synthesis (per reaction)

1. Anneal the TargetAmp T7-Oligo(dT) Primer A to the RNA sample.

x	µl	RNase-Free Water
x	µl	Total RNA sample
1	µl	TargetAmp T7-Oligo(dT) Primer A
3	µl	Total reaction volume

2. Incubate at 65°C for 5 minutes, then chill on ice for 1 minute, then quick spin the sample.
3. Add 2 µl of the following First-strand cDNA Synthesis Master Mix.

1.5	µl	TargetAmp Reverse Transcription PreMix-SS
0.25	µl	DTT
0.25	µl	SuperScript III Reverse Transcriptase (200 U/µl)
2	µl	Total reaction volume

4. Incubate at 50°C for 30 minutes.

Second-strand cDNA Synthesis (per reaction)

5. Add 5 µl of the following Second-strand cDNA Synthesis Master Mix.

4.5	µl	TargetAmp DNA Polymerase PreMix-SS 1
0.5	µl	TargetAmp DNA Polymerase-SS 1
5	µl	Total reaction volume

6. Incubate at 65°C for 10 minutes,
   then quick spin the sample,
   then incubate at 80°C for 3 minutes,
   then quick spin the sample,
   then chill on ice.

***In Vitro* Transcription of aRNA** (per reaction)

7. Add 40 µl of the following *In Vitro* Transcription Master Mix.

13.6	µl	RNase-Free Water
4	µl	TargetAmp T7 Transcription Buffer
3.6	µl	ATP
3.6	µl	CTP
3.6	µl	GTP
3.6	µl	UTP
4	µl	DTT
4	µl	TargetAmp T7 RNA Polymerase
40	µl	Total reaction volume

8. Incubate at 42°C for 4 hours.
9. Add 2 µl of RNase-Free DNase I, incubate at 37°C for 15 minutes.

aRNA Purification

The purification column to use is dependent upon the expected yield of aRNA. See Part D.

5. Appendix 1 The TargetAmp 1-Round aRNA Amplification Kit 103 Control Reaction

The TargetAmp kit provides 400 ng of total human HeLa RNA at a concentration of 40 ng/µl.

Required for the TargetAmp Control Reaction

Component Name	Tube Label	Cap Color
HeLa Total RNA Control (40 ng/µl)	HeLa Total RNA	Clear
TargetAmp T7-Oligo(dT) Primer A	T7-Oligo(dT) Primer A	Red
RNase-Free Water	RNase-Free Water	Clear

1. Thaw the HeLa Total RNA Control on ice.
2. Anneal the TargetAmp T7-Oligo(dT) Primer A to the HeLa Total RNA Control. The standard control reaction utilizes 100 ng of the HeLa Total RNA Control. If using less Control RNA, make up the volume of the annealing reaction with RNase-Free Water.

2.5	µl	HeLa Total RNA Control (100 ng)
1	µl	TargetAmp T7-Oligo(dT) Primer A
3.5	µl	Total reaction volume

3. Incubate the reaction at 65°C for 5 minutes in a water bath or thermocycler. While the reaction incubates, quick-freeze the HeLa Total RNA Control (40 ng/µl; for example in a dry ice/ethanol bath) and return it to –70°C to –80°C storage.
4. Cool the annealing reaction on ice for at least 1 minute. Centrifuge the tube for 5-10 seconds to bring the sample to the bottom of the tube.
5. Continue the Control Reaction as described beginning in Part A, Step 4.

6. Additional TargetAmp aRNA Amplification Kits & Selection Guide

	TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101	TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 105	TargetAmp™ Nano Labeling Kit for Illumina® Expression BeadChip®
Starting Total RNA	25-500 ng	25-500 ng	25-500 ng
Reverse Transcriptase(s) Used	SuperScript® III (provided by the user)	SuperScript® III (provided by the user)	SuperScript® III (provided by the user)
Fold Amplification	> 5,000	> 5,000	> 5,000
Time Required	1 Day	1 Day	1 Day
End Product	Aminoallyl-aRNA	Biotin-aRNA	Biotin-aRNA

	TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0	TargetAmp™ 2-Round aRNA Amplification Kit 2.0	TargetAmp™ 2-Round Biotin-aRNA Amplification Kit 3.0
Starting Total RNA	10-500 pg	10-500 pg	50-500 pg
Reverse Transcriptase(s) Used	SuperScript® III & SuperScript® II (provided by the user)	SuperScript® III & SuperScript® II (provided by the user)	SuperScript® III & SuperScript® II (provided by the user)
Fold Amplification	>5,000,000	>5,000,000	>5,000,000
Time Required	2 Days	1 Day	2 Days
End Product	Aminoallyl-aRNA	Unlabeled-aRNA	Biotin-aRNA

TargetAmp™ 1-Round Aminoallyl-aRNA Amplification Kit 101

TAA1R4910 10 Reactions

TAA1R4924 24 Reactions

This kit will amplify Poly(A) RNA by >5,000 fold from as little as 25 ng of total cellular RNA. The kit produces aminoallyl-aRNA and is optimized for use of SuperScript III Reverse Transcriptase (provided by the user).

TargetAmp™ 1-Round Biotin-aRNA Amplification Kit 105

TAB1R80510 10 Reactions

TAB1R80524 24 Reactions

This kit will amplify Poly(A) RNA by >5,000 fold producing microgram amounts of Biotin-aRNA from as little as 25 ng of total cellular RNA. The kit is optimized for use with SuperScript III Reverse Transcriptase (provided by the user).

TargetAmp™ Nano Labeling Kit for Illumina® Expression BeadChip®

TAN07924 24 Reactions

This kit will produces microgram amounts of Biotin-aRNA from as little as 25 ng of total cellular RNA for hybridization to Illumina® Expression BeadChips. The kit is optimized for use with SuperScript III Reverse Transcriptase (provided by the user).

TargetAmp™ 2-Round Aminoallyl-aRNA Amplification Kit 1.0

TAA2R4910 10 Reactions

TAA2R4924 24 Reactions

This kit will amplify Poly(A) RNA by >5,000,000 fold producing microgram amounts of aminoallyl-aRNA from as little as 10 pg of total cellular RNA. The kit is optimized for use with SuperScript III and SuperScript II Reverse Transcriptases (provided by the user).

TargetAmp™ 2-Round aRNA Amplification Kit 2.0

TAU2R5110 10 Reactions

TAU2R51224 24 Reactions

This kit will amplify Poly(A) RNA by >5,000,000 fold producing microgram amounts of unlabeled aRNA from as little as 10 pg of total cellular RNA. The kit is optimized for use with SuperScript III and SuperScript II Reverse Transcriptases (provided by the user).

TargetAmp™ 2-Round Biotin-aRNA Amplification Kit 3.0

TAB2R71010 10 Reactions

TAB2R71024 24 Reactions

This kit will amplify Poly(A) RNA by >5,000,000 fold producing microgram amounts of biotin-aRNA from as little as 50 pg of total cellular RNA. The kit is optimized for use with SuperScript III and SuperScript II Reverse Transcriptases (provided by the user).

7. Related Products

Reverse Transcriptase

MonsterScript™ Reverse Transcriptase

MSTA5110 10 Reactions

MSTA5124 24 Reactions

MonsterScript Reverse Transcriptase is a thermostable reverse transcriptase that completely lacks RNase H activity. The enzyme is highly efficient at producing full-length cDNA from RNA templates up to or greater than 15 kb.

Kits for Isolating Total Cellular RNA and mRNA

ArrayPure™ Nano-scale RNA Purification Kit

MPS04050 50 Purifications

The ArrayPure Kit provides all reagents needed to purify total RNA from 1-10,000 eukaryotic cells without the use of organic solvents such as phenol or columns.

MasterPure™ RNA Purification Kit

MCR85102 100 Purifications

The MasterPure RNA Purification Kit provides all reagents needed to purify total RNA from >10,000 eukaryotic cells without the use of organic solvents such as phenol or columns.

mRNA-ONLY™ Eukaryotic mRNA Isolation Kit

MOE51010 10 Purifications

MOE51024 24 Purifications

The mRNA-Only Eukaryotic mRNA Isolation Kit provides simple and effective isolation of eukaryotic mRNA that is substantially free of ribosomal RNA in 1 hour. The kit does not use oligo(dT), columns or resins.

mRNA-ONLY™ Prokaryotic mRNA Isolation Kit

MOP51010	10 Purifications
MOP51024	24 Purifications

The mRNA-Only Prokaryotic mRNA Isolation Kit provides simple and effective isolation of prokaryotic mRNA that is substantially free of ribosomal RNA in 1 hour.

Aminoallyl-aRNA Labeling Reagents

Biotin-X-X-NHS

BXX51005	5 x 2.5 mg vials
BXX51010	10 x 2.5 mg vials

Biotin-X-X-NHS can be used to rapidly and cost-effectively label aminoallyl-aRNA and aminoallyl-DNA with biotin for use in DNA microarrays or other biotin-probe applications.

Aminoallyl-UTP

AAU5202	2.5 µmol @ 50 mM

Aminoallyl-UTP is supplied in a convenient 50 mM solution.

8. Reference

1. Van Gelder, R. N. *et al.*, (1990) *Proc. Natl. Acad. Sci. USA* **87** (5), 1663.

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