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Product Information

Complete Whole Transcriptome Amplification Kit

Catalog Number **WTA2**
Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

WTA2, a Whole Transcriptome Amplification (WTA) method, allows for representative amplification of nanogram quantities of total RNA in less than 4 hours without 3′-bias. The resulting microgram quantities of product generated from tissue, cultured cells, formalin-fixed samples, or serum are suitable for downstream applications such as qPCR and microarray analyses. The Complete WTA Kit provides everything needed for amplification including the amplification enzyme.

The WTA process involves two steps. In the first step, sample RNA is reverse transcribed with primers composed of a semi-degenerate 3′ end and a universal 5′ end. As polymerization proceeds, displaced single strands serve as new templates for primer annealing and extension. The resultant cDNA library, composed of random, overlapping fragments flanked by universal end sequence, is then amplified by PCR with the universal primer to produce WTA product. Product size ranges from 100–1000 bases when amplifying intact RNA, and typically smaller for degraded RNA.

Components

Description	Catalog Number	10 RXN	50 RXN
Library Synthesis Buffer	L9418	25 µL	125 µL
Library Synthesis Solution	L9293	25 µL	125 µL
Library Synthesis Enzyme	L9543	20 µL	100 µL
Amplification Mix	A6731	375 µL	1.875 mL
10mM dNTP Mix	D7295	0.2 mL	0.5 mL
Nuclease-Free Water	W4502	5 mL	20 mL
Amplification Enzyme	A6856	37.5 µL	187.5 µL

Materials and Reagents Required but Not Provided

- Thermal cycler
- RNA to be amplified
- Spectrophotometer
- 0.2 mL or 0.5 mL thin-walled PCR tubes or PCR multiwell plate
- Dedicated pipettes
- PCR pipette tips
- SYBR® Green I nucleic acid gel stain, Catalog Number S9430

Precautions and Disclaimer

This product is for R&D use only, not for drug, household, or other uses. Please consult the Material Safety Data Sheet for information regarding hazards and safe handling practices.

Storage/Stability

All components should be stored at –20 °C. When thawed for use, components should be kept on ice. Stability of the WTA Library Synthesis Enzyme will be affected if stored warmer than –20 °C or allowed to remain for long periods at temperatures over 4 °C. RNA sample (not included) should be thawed on ice.

Procedure

Following this procedure should produce 25-40 μg of WTAmplicon, starting from 5-25 ng of high quality total RNA. Typically, 5- to 10-fold more input RNA is recommended when isolated from FFPE tissues. Reactions can be scaled up or down to accommodate preparation of necessary quantities of final product.

Library Synthesis Reaction

1. Thaw the Library Synthesis Buffer, Library Synthesis Solution, Library Synthesis Enzyme and Nuclease-Free Water. Mix Library Synthesis Buffer and Library Synthesis Solution thoroughly. Dissolve any precipitate in these solutions by briefly heating at 37 °C, with thorough mixing.

2. To at least 25 ng of total RNA (5 ng per 75 μL reaction, step 10) add:
   a. 2.5 μL Library Synthesis Solution
   b. Nuclease-Free Water to a total of 16.6 μL

   Note: At least 250 ng of degraded total RNA, e.g., from FFPE tissues, is recommended per 375 μL reaction (50 ng per 75 μL reaction, step 10).

3. Mix and incubate in a thermocycler programmed for 70 °C for 5 minutes then 18 °C.

4. To the cooled-primed RNA immediately add the following (individually or premixed)
   a. 2.5 μL Library Synthesis Buffer
   b. 3.9 μL Water
   c. 2 μL Library Synthesis Enzyme

5. Incubate in a thermal cycler using the following parameters:

   18º C for 10 minutes
   25º C for 10 minutes
   37º C for 30 minutes
   42º C for 10 minutes
   70º C for 20 minutes
   4º C

6. Consolidate any condensation by centrifugation and mixing.

Amplification Reaction

7. Thaw the Amplification Mix and 10mM dNTP Mix.

8. Prepare the following master mix
   a. 301 μL Nuclease-Free Water
   b. 37.5 μL Amplification Mix
   c. 7.5 μL WTA dNTP Mix
   d. 3.75 μL Amplification Enzyme

Note: For real-time PCR, deduct the volumes of a reference dye and 3.75 μL of a 1:1000 dilution (in Nuclease–Free Water) of SYBR® Green stain from the water volume. Add SYBR® Green dilution to master mix immediately before dispensing. Prepare a fresh dilution for each experiment,

9. Add the entire Library Synthesis reaction from Step 6 (25 μL) to the master mix solution from Step 8 and mix.

10. Divide the above (step 9) into five 75 μL reactions. (A reaction volume of < 75 μL, for the last aliquot, is not critical.) Incubate in a thermal cycler using the following parameters:

    94° C for 2 minutes.
    17 cycles x (94° C for 30 seconds, 70° C for 5 minutes)

Note: The optimal number of amplification cycles varies with template amount and quality. Seventeen cycles is recommended for 5 ng of high quality RNA or 50 ng of FFPE RNA. Due to variations in the level of degradation (e.g., RNAs isolated from FFPE tissues), some RNA samples may require higher input quantities and/or more cycles. Optimal cycle number is achieved by proceeding 2–3 cycles beyond the amplification "plateau", as observed with real-time quantitative PCR.

11. After cycling is complete, maintain the reactions at 4 °C or store at –20 °C until ready for analysis or purification. The stability of WTA DNA is equivalent to genomic DNA stored under the same conditions.

12. For removal of residual primers and nucleotides, use any standard PCR purification kit or equivalent methods for purification of double and single-stranded DNA.

13. Purified DNA is quantified by measuring absorbance. 1 A_{260} unit is equivalent to 50 ng/μL DNA. Measurement techniques such as PicoGreen® dye will often underestimate the actual WTA DNA yield, since single stranded DNA may be present following amplification.

Product Profile
All lots are tested to meet performance specification for amplification of high quality human total RNA. Performance testing includes monitoring amplification in real-time to determine efficiency of template utilization, quantifying product yields by UV absorbance and gene specific qPCR.

References

1. Hertzberg, M, *et al*. cDNA microarray analysis of small plant tissue samples using a cDNA tag target amplification protocol. *Plant J*, **25**, 585-91 (2001).
2. Iscove, N.N., *et al*. Representation is faithfully preserved in global cDNA amplified exponentially from sub-picogram quantities of mRNA. *Nat. Biotechnol*, **20**, 940-3 (2002).
3. Klur, S, *et al*. Evaluation of procedures for amplification of small–size samples for hybridization on microarrays. *Genomics*, **83**, 508-17 (2004).
4. Nagy, Z.B, *et al*. Real-time polymerase chain reaction-based exponential sample amplification for microarray gene expression profiling. *Anal. Biochem*, **337**, 76-83.

Troubleshooting Guide

Observation	Potential Cause	Recommended Solution
Low yield	Sample RNA quality (degraded or impure)	Titrate input RNA quantity up to 300 ng
		Evaluate different RNA preparation methods
		Increase PCR cycles
		Monitor amplifications on real-time instrument to determine optimal PCR cycle
		Pool multiple reaction product of degraded or impure samples
	Quantified using PicoGreen	Determine yield by UV absorbance
	Didn't purify single-stranded or small products	Use a kit that purifies double and single-stranded DNA
		Use a kit capable of purifying 100 bp PCR products
Rare transcripts not efficiently incorporated during library amplification	Insufficient RNA input	Increase RNA quantity

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