

Product Information

TransPlex® Whole Transcriptome Amplification Kit

Catalog Number **WTA1**
 Storage Temperature –20 °C

TECHNICAL BULLETIN

Product Description

TransPlex®, a Whole Transcriptome Amplification (WTA) method, allows for representative amplification of nanogram quantities of total RNA in less than 4 hours without 3'-bias. Microgram quantities of amplification product generated from tissue, cultured cells, formalin-fixed samples, or serum are suitable for downstream applications such as qPCR and microarray analyses.

The WTA process involves two steps. In the first step, sample RNA is reverse transcribed with non-self-complementary primers composed of a quasi-random 3' end and a universal 5' end. As polymerization proceeds, displaced single strands serve as new templates for primer annealing and extension. The resultant Omniplex® cDNA library, composed of random, overlapping 100–1000 base fragments flanked by universal end sequence, is then amplified by PCR with the universal primer to produce WTA product.

Components

Description	Catalog Number	10 RXN	50 RXN
WTA Library Synthesis Buffer	L7168	30 µL	135 µL
WTA Library Stabilization Solution	L7043	30 µL	135 µL
WTA Library Synthesis Enzyme	L6918	15 µL	60 µL
WTA Amplification Master Mix	A0356	405 µL	2 × 1.0 mL
WTA dNTP Mix	D6193	85 µL	405 µL
Nuclease-Free Water	W4765	5.0 mL	4 × 5.0 mL

Materials and Reagents Required but Not Provided

- An antibody inactivated hot-start Taq DNA Polymerase*
- 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**

*Some Taq DNA polymerases can lead to the formation of product in no-template controls. This no-template product will not contain genes of interest if probed using PCR or hybridization techniques.

**Nuclease-free

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.

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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

Library Preparation

1. Thaw WTA Library Synthesis Buffer and WTA Library Stabilization Solution on ice and mix thoroughly. Dissolve any precipitate in these solutions by briefly heating at 37 °C and mix thoroughly.
2. To 5–300 ng of total RNA, add, individually or pre-mixed, the following
 - 2.5 µL WTA Library Synthesis Buffer
 - 2.5 µL WTA Library Stabilization Solution
 - Nuclease-free water for a total volume of 24 µL.
3. Mix by pipetting and incubate at 70 °C for 5 minutes.
4. Cool reaction immediately on ice and spin down any condensation by centrifugation.
5. Add 1 µL of WTA Library Synthesis Enzyme and mix by pipetting.
6. Incubate in thermal cycler using the following parameters:
 - 24° C for 15 minutes
 - 42° C for 2 hours
 - 95° C for 5 minutes
7. Chill reaction immediately on ice. Spin down any condensation by centrifugation.

Amplification

8. Thaw WTA Amplification Master Mix and dNTP Mix on ice and mix thoroughly.
9. Prepare the following WTA Amplification Mix:*
 - 300 µL Nuclease-free water
 - 37.5 µL WTA Amplification Master Mix
 - 7.5 µL dNTP Mix
 - 12.5 units of antibody inactivated hot-start Taq DNA Polymerase

*For real-time PCR, include a reference dye as necessary and 3.75 µL of a 1:1000 dilution of SYBR® Green stain.

10. Divide the library, placing 5 µL aliquots in individual tubes or wells.
 11. Add 70 µL of WTA Amplification Mix to each aliquot and mix well. Incubate in thermal cycler using the following parameters:
 - 95° C for 3 minutes.
 - 17 cycles* x (94° C for 20 seconds, 65° C for 5 minutes)
- *Optimal cycle number varies with template amount and quality. 17 cycles is recommended for 5 ng of high quality RNA. Optimal cycle number is achieved by proceeding 2–3 cycles into the amplification “plateau”.
12. 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.
 13. For removal of residual primers and nucleotides, use any standard PCR purification kit or equivalent methods for purification of double and single-stranded DNA.
 14. Purified DNA is quantified by measuring absorbance. 1 A₂₆₀ 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 generated during 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 and quantifying product yields by UV absorbance.

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.

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 Polymerase Chain Reaction (PCR) is covered by patent owned by Roche Molecular Systems and F Hoffmann-La Roche Ltd

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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
Rare transcripts not efficiently incorporated during library amplification	Insufficient RNA input	Use a kit that purifies double and single-stranded DNA
		Use a kit capable of purifying 100 bp PCR products
	Didn't use a hot start Taq DNA Polymerase	Use an antibody inactivated hot-start Taq DNA Polymerase

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