TransPLEX® C-WTA Kit Quick Protocol

The TransPLEX® C-WTA Kit amplifies total RNA to produce over 5 micrograms of DNA products suitable for both microarray and qPCR analyses in 5 hours.

TransPLEX C-WTA has two steps, library synthesis and amplification. To synthesize the library, sample RNA is incubated with a reverse transcriptase and non-self-complementary primers comprised of a quasi-random 3' end and a universal 5' end. When annealed primers are extended by polymerase, displaced single strands are generated which become new templates for primer annealing and extension. This process creates a TransPLEX library comprised of random, overlapping fragments flanked by a universal end sequence. Universal-primer PCR is then used to amplify the TransPLEX library and produces C-WTA products.

For more information, please visit www.rubicongenomics.com/products/TransPLEX/.

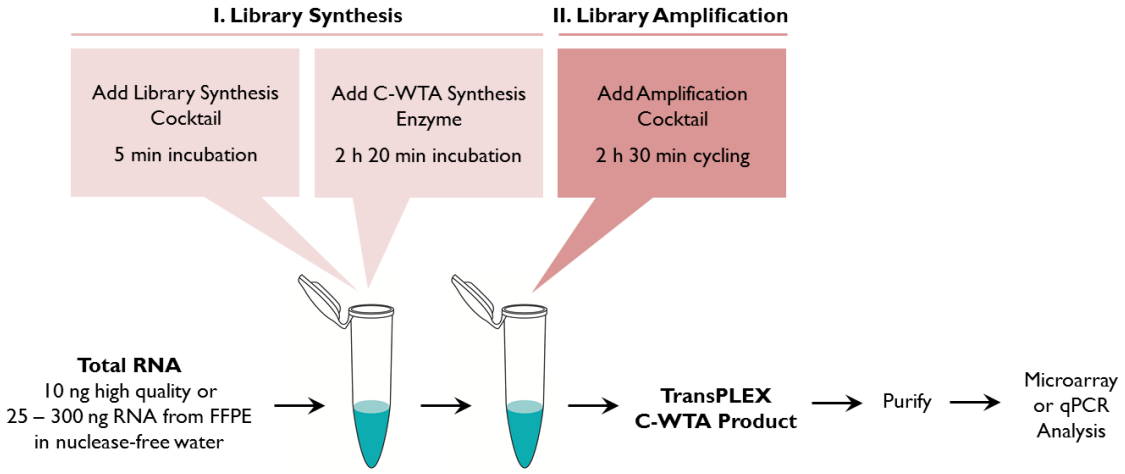
Storage: Store kit at –20°C upon arrival.

Technical support: Call (734)-677-4845 (9AM-5:30PM Eastern Time) or contact support@rubicongenomics.com.

Kit Contents

Name	Part Number	Cap Color	100 Reactions CAT. NO. RC20100
C-WTA Synthesis Buffer	RC20100-01	Green	1 Tube
C-WTA Stabilization Solution	RC20100-02	Yellow	1 Tube
C-WTA Synthesis Enzyme	RC20100-03	Blue	1 Tube
C-WTA Amplification Buffer	RC20100-04	White	1 Tube
C-WTA Amplification Enzyme	RC20100-05	Red	1 Tube
Quick Protocol	RC20100-06		

Workflow



A. Notes Before Starting

1. **Preparation of Master Mixes:**
   - Thaw the C-WTA Stabilization Solution on ice. Briefly vortex component prior to use.
   - Thaw the C-WTA Synthesis and Amplification Buffers at room temperature and transfer to ice. Briefly vortex components prior to use.
   - Transfer C-WTA Synthesis Enzyme and C-WTA Amplification Enzyme to ice just before use.
2. **User Supplied Materials:**
   - Thermal cycler (Real-time instrument recommended)
   - Nuclease-free water
   - PCR tubes or 96-well PCR plate
   - PCR plate seals
   - Low-binding barrier tips
   - QIAquick® PCR Purification Kit (QIAGEN, CAT. NO. 28104 or 28106)
   - 3M sodium acetate (pH 5.2)

B. Quick Protocol

I. Library Synthesis

1. Add Nuclease-Free Water to total RNA (10 ng of high quality or 25 – 300 ng of RNA from FFPE) to achieve a total sample volume of 16.5 µL in a PCR tube or well.
2. Thaw the C-WTA Synthesis Buffer at room temperature with intermittent vortexing for 15 – 20 min or until the white precipitate is no longer visible and the solution is homogenous. Briefly spin and return to ice prior to use.
3. Combine the following Library Synthesis Cocktail components and pipet to mix.

Library Synthesis Cocktail		
Component	**Cap Color**	**Volume/Rxn**
C-WTA Synthesis Buffer	Green	5.0 µL
C-WTA Stabilization Solution	Yellow	2.5 µL
Total Volume		7.5 µL

4. Add 7.5 µL of freshly prepared Library Synthesis Cocktail to the RNA sample and mix by pipet.
5. Incubate sample in a thermal cycler as follows:

Temperature	Time	No. of Cycles
70°C	5 min	1
4°C	Hold	1

6. Briefly centrifuge sample to collect liquid at bottom of tube/well and place sample on ice.
7. Add 1 µL of C-WTA Synthesis Enzyme (blue cap) to sample for a total of volume of 25 µL and mix by pipet.
8. Incubate sample in a thermal cycler as follows to produce a TransPLEX Library:

Temperature	Time	No. of Cycles
24°C	15 min	1
42°C	2 h	1
95°C	5 min	1
4°C	Hold	1

9. Briefly centrifuge the Library and transfer a single 5 µL aliquot of the Library to a new tube/well to prepare for the Library Amplification procedure.

II. Library Amplification

1. Thaw the C-WTA Amplification Buffer at room temperature. If a precipitate is observed, incubate the tube at 37°C for 3 min (or until no precipitate is visible) with intermittent vortexing. Briefly spin and return the tube to ice prior to use.
2. Combine the following Amplification Cocktail components and vortex briefly to mix.

Library Amplification Cocktail		
Component	**Cap Color**	**Volume/Rxn**
Nuclease-Free Water		54 µL
C-WTA Amplification Buffer	White	15 µL
C-WTA Amplification Enzyme	Red	1 µL
Total Volume		70 µL

3. Add 70 µL of freshly prepared Amplification Cocktail to the 5 µL Library aliquot (prepared in Step I.9) and mix by pipet.
4. Amplify sample according to thermal cycler program below:

Temperature	Time	No. of Cycles
95°C	2 min	1
95°C	20 s	22
65°C	5 min	
4°C	Hold	1

5. Briefly centrifuge and immediately store the amplified product at –20°C or proceed to purifying the amplified products.

Purifying Amplified Products

The C-WTA products should be purified to remove residual primers and nucleotides which may interfere with downstream processes, such as labeling reactions.

The C-WTA product should be purified on one QIAquick column as instructed in the QIAquick® PCR Purification Kit (QIAGEN, CAT. NO. 28104 or 28106), with the following modifications to the standard protocol:

1. Add 375 µL of Buffer PB and 10 µL of 3M sodium acetate (pH 5.2) to the 75 µL product and mix by vortexing before loading the sample on a column.
2. Elute purified product in 50 µL of TE Buffer (pH 8.0), allowing column to stand for 2 minutes at room temperature after adding TE before centrifuging.

The purified, amplified product may be stored at –20°C.

Quantifying Amplified Products

UV absorbance (A260) should be used to quantify purified products, using the conversion of 1 OD = 50 µg/mL.

Approximately 10 – 15 µg of amplified product should be generated from 10 ng of Universal Human Reference RNA (Agilent, CAT. NO. 740000) sample.