

## Frequently Asked Questions

### WT-Ovation™ RNA Amplification (cat # 2210-24)



#### **Q1. What materials are provided with the WT-Ovation™ RNA Amplification System?**

The WT-Ovation™ System provides all necessary buffers, primers and enzymes for first strand synthesis, second strand synthesis and amplification, yielding cDNA. The kit also provides nuclease-free water.

#### **Q2. What equipment is required or will be useful?**

Required equipment includes a microcentrifuge, pipettes, vortexer and a thermal cycler. An O.D. spectrophotometer and an Agilent Bioanalyzer will be useful.

#### **Q3. What additional consumables does the user need?**

None. For the optional purification of amplified cDNA, see user guide for validated purification products and procedures.

#### **Q4. Do I need to use high quality total RNA?**

Use of lower quality RNA may result in poor performance. One approach to determining RNA quality is the Agilent Bioanalyzer's RNA Integrity Number (RIN). Clean RNA with a RIN score of greater than 7 should amplify well.

#### **Q5. Can I do reactions in smaller batches than 8?**

We recommend 3 batches of 8 reactions. Smaller batch sizes may result in fewer than 24 reactions in total.

#### **Q6. Where in my target sequence can I design my QPCR primers?**

The WT-Ovation™ system does not have a 3 prime bias and therefore primers can be designed at any location within the mRNA. In order to avoid QPCR interference from possible genomic DNA contamination, we recommend treating your RNA with DNase and designing your amplicons to span an intron.

#### **Q7. Is the WT-Ovation™ System 3 prime biased?**

No, this product is different from the Ovation™ RNA Amplification System in that the first strand cDNA is primed with random hexamers as well as a 3 prime primer.

#### **Q8. How much total RNA do I need for amplification?**

We recommend staying within the range of 5 to 50 ng total RNA starting material. Amounts greater than 50 ng may produce variable results.

#### **Q9. How much cDNA can I expect from a single reaction?**

You should expect 1.5 to 4 µg of cDNA from 5 to 50 ng total RNA starting material.

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**Q10. Is the cDNA yield dependent upon the quantity of input total RNA?**

Yes, the more RNA into the assay, the more yield recovered. However at inputs of above 50 ng, the yields become variable without increasing.

**Q11. What is the amplification efficiency of the WT-Ovation™ System?**

Based on QPCR on a variety of genes, an average amplification efficiency of 1500-fold is observed.

**Q12. What is the dynamic range of input mRNA that is linearly amplified with the WT-Ovation™ System?**

Our studies demonstrate linear amplification over 6 orders of magnitude starting with transcripts present in as low as 20 copies in a sample.

**Q13. What size cDNA is generated by the WT-Ovation™ System?**

On a Bioanalyzer, using the RNA 6000 size markers, the average size of the amplified cDNA is 375 bases. More than 50% of the product is greater than 320 bases in length.

**Q14. Can the WT-Ovation™ System amplify DNA?**

The Ovation™ system is designed to amplify mRNA, not DNA.

**Q15. Can I use the WT-Ovation™ System on bacterial RNA samples?**

The WT-Ovation™ theoretically will work with some bacterial RNAs. However, the kit has not been optimized for this purpose.

**Q16. Are there any tissues that will not work with the WT-Ovation™ System?**

We have not encountered any good quality, clean RNA samples containing poly (A) + RNA that will not work with the Ovation™ System.

**Q17. Has NuGEN performed reproducibility studies on the WT-Ovation™ System?**

Yes. Sample to sample, lot to lot, and operator to operator reproducibility studies are routinely conducted.

**Q18. Does the Ovation™ System generate product in the absence of RNA input?**

No significant product is generated in the absence of input RNA.

**Q19. How many rounds of amplification are performed with the WT-Ovation™ System?**

The WT-Ovation™ System performs a single round of amplification.

**Q20. Can I use the WT-Ovation™ System for archiving cDNA?**

Amplified cDNA maybe stored at -20°C for at least several months. Long term stability tests are in progress.

**Q21. Do I need to order specific primers for the amplification?**

No. The DNA/RNA primers provided in the WT-Ovation™ System are universal.

**Q22. Do I have to use your DNA/RNA primers?**

The WT-Ovation™ System will not work properly with other primers.

**Q23. What are the incubation temperatures for each step?**

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First strand primer annealing = 65 °C  
First strand synthesis = 25 °C and 42 °C  
Second strand synthesis = 25 °C and 50 °C  
Post second strand Enhancement = 37 °C  
SPIA™ amplification = 47 °C

**Q24. If I choose to purify my product, what method do you recommend?**

We suggest purification with a Zymo Research DNA Clean & Concentrator™-25, the NucleoSpin® Extract I kit, the QIAquick® PCR purification kit, or the Agencourt AMPure® magnetic beads. See the Additional Equipment, Reagents, and Labware section of this user guide for order information and Appendix B for procedures.

**Q25. Should I purify the cDNA if I chose to determine the concentration?**

Yes, the primers and reagents present in the amplified cDNA will interfere with accurate quantitation. Other details on measuring the concentration of cDNA are included in the user guide.

**Q26. How do I measure my cDNA product?**

Measure the amplified product as suggested in Appendix C.

**Q27. Where can I safely stop in the protocol?**

We do not recommend stopping at any stage of the protocol.

**Q28. How many QPCR reactions will I get from one WT-Ovation™ amplification?**

The number of QPCR reactions depends on the abundance level of the genes being interrogated. For medium to high copy genes, the cDNA may be diluted as much as 400-fold, enough for hundreds of QPCR reactions. For very low copy genes you will need to use more cDNA per reaction. The user will need to determine how much cDNA to use per reaction depending on the abundance of the gene being interrogated.

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