

# Frequently Asked Questions

## Ovation™ RNA Amplification System V2 (Cat # 3100-12, 3100-60)



*This product is the modular version of amplification components from NuGEN's Ovation™ Biotin System (Cat.# 2300)*

### **Q1. What materials are provided with the Ovation™ RNA Amplification System V2?**

The Ovation™ RNA Amplification System V2 provides all necessary buffers, primers and enzymes for first strand synthesis, second strand synthesis and amplification.

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

Required equipment includes a microcentrifuge, pipettes, vortexer and a thermal cycler. A UV/Vis spectrophotometer and an Agilent Bioanalyzer will be useful and a real time PCR system may be used for quality control.

### **Q3. What additional consumables are required or useful for the Ovation™ RNA Amplification System V2?**

Only lab consumables are required, see page 5 for a listing. For the optional cDNA purification, the Zymo Research DNA Clean and Concentrator™-25 columns are needed (Zymo Research, Cat. #D4005).

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

We recommend staying within the specified range of 5 to 100 ng of total RNA starting material. We often suggest 20 ng input as an appropriate starting point. Input greater than 100 ng may adversely affect amplification.

### **Q6. What is the dynamic range of input mRNA copies that are linearly amplified?**

Our studies demonstrate linear amplification of transcripts present at 100 to 100,000,000 copies in a 5 ng sample of HeLa RNA. Different input amounts of RNA from different tissues may affect the lower limit of detection.

### **Q7. Can I use a total RNA input of less than 5 ng?**

The Ovation™ RNA Amplification System V2 has been validated for total RNA input amounts of 5 to 100 ng. Using input quantities outside the recommended range will affect quality and quantity of resulting cDNA.

### **Q8. Can I omit quantitation of input RNA?**

We do not recommend omitting quantitation of input RNA. However if sample size constraints absolutely prohibit quantitation, you may save a small aliquot (2 µl) following the second strand cDNA synthesis, in order to retrospectively estimate the starting input RNA concentration by quantitative RT-PCR. (Refer to Technical Note 1 for more detail on this procedure).

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**Q9. Can I use mRNA instead of total RNA as starting material?**

Purified poly(A) RNA has been successfully used as input to the Ovation™ System. It will be necessary to use much lower amounts of input mRNA, comparable to mRNA present in 5-100 ng of a total RNA sample from the same tissue.

**Q10. Is the Ovation™ RNA Amplification System V2, 3 prime biased?**

Since the Ovation™ System primes the poly A tail of transcripts, it is 3 prime biased, resulting in coverage to a range of 1500 bases from the 3' poly A tail.

**Q11. How much cDNA yield can I expect from one reaction of Ovation™ amplification?**

For a standard reaction the expected yield is 4-7 µg of amplified cDNA.

**Q12. Is the cDNA yield dependent upon the quantity of input total RNA?**

The total yield of cDNA is not directly dependent upon input RNA amount due to upper limit constraints on cDNA production in the reaction.

**Q13. What is the amplification efficiency of the Ovation™ RNA Amplification System V2?**

Based on qPCR results of a collection of housekeeping genes, amplification efficiency ranges from 1,000 to 10,000 fold or higher depending on the input amount.

**Q14. What is the size range of cDNA generated by the Ovation™ System?**

As measured with an Agilent Bioanalyzer, the majority of amplified SPIA™ cDNA is between 200 bases and 2000 bases in length. After fragmentation, 80% of product falls below 200 bases with an average peak at 85 bases.

**Q15. Has NuGEN performed reproducibility studies on the Ovation™ System?**

Our studies have included sample to sample, lot to lot, and operator to operator reproducibility.

**Q16. Can the Ovation™ System kits be used for amplification of DNA?**

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

**Q17. Can I use the Ovation™ System on prokaryotic RNA samples?**

The Ovation™ System relies on the presence of a poly A tail for priming. Therefore, it will not amplify most prokaryotic RNA.

**Q18. Are there any tissues that will not work with the Ovation™ System?**

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

**Q19. Does the Ovation™ System generate product in a no-RNA reaction?**

As with most amplification systems, non-specific product is generated using the Ovation™ RNA Amplification System V2 in the absence of input template. Array and qPCR analysis show these amplification products to be non-specific.

**Q20. How many rounds of amplification are performed with the Ovation™ System?**

The Ovation™ System performs a single round of amplification in less than 4 hours. Our products are designed to provide high sensitivity through robust amplification without necessitating a second round of amplification.

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

The chimeric DNA/RNA primers provided with the Ovation™ System kits are universal, and there is no need for additional primers.

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

The Ovation™ System was designed and optimized to work with the primers provided. The use of other primers with the Ovation™ System is not supported.

**Q23. Why is the purification of cDNA step optional?**

cDNA purification is required for microarray applications. cDNA for fragmentation and labeling reactions and array analysis must be purified to allow for accurate Quantitation of cDNA as well as ensure proper performance of the FL-Ovation™ cDNA Biotin Module V2.

If the amplified cDNA is to be used for qPCR analysis alone, then purification is optional.

Purification will enable mass normalization of cDNA input into the qPCR reaction potentially reducing variability.

**Q24. Can I fragment the cDNA?**

Yes, cDNA fragmentation and labeling can be achieved using the NuGEN FL-Ovation™ cDNA Biotin Module V2 to prepare target for analysis on Affymetrix GeneChip® arrays.

**Q25. Can I use the Ovation™ RNA Amplification System V2 for archiving cDNA?**

cDNA may be stored following purification for at least 6 months. Longer term stability tests are in progress.

**Q26. What are the recommended storage conditions for amplified cDNA?**

The amplified cDNA may be stored at -20°C. Ensure the vials are well sealed and avoid multiple freeze thaw cycles.

**Q27. What types of arrays work with the cDNA generated with the Ovation™ RNA Amplification System V2?**

This cDNA generated with the Ovation™ RNA Amplification System V2, fragmented and labeled using FL-Ovation™ cDNA Biotin Module V2 has been validated on Affymetrix GeneChip® arrays.

**Q28. To proceed to fragmentation and labeling how much cDNA should I have?**

The required input for FL-Ovation™ cDNA Biotin Module V2 is 3.75 µg of amplified cDNA to prepare target for Affymetrix GeneChip® arrays.

**Q29. How much labeled cDNA should I hybridize to a GeneChip® array?**

For standard arrays you will need to hybridize 3.75 µg of fragmented and labeled cDNA, when using the FL-Ovation™ cDNA Biotin Module V2 product.

**Q31. For quantitative real time PCR applications, what is the optimal distance from the 3 prime poly A tail for design of primer probe sets?**

Due to the amplification mechanism of the Ovation™ System, we recommend primer/probe sets to be designed within the first 1,500 bases from the poly A tail.

**Q32. What are the incubation temperatures for each step?**

First strand primer annealing = 65°C

First strand synthesis = 48°C

Second strand synthesis = 37°C

SPIA™ amplification = 48°C

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**Q33. Where can I safely stop in the protocol?**

You may safely stop after cDNA amplification or purification and store the cDNA at -20°C.

**Q34. How can I ensure good yields at the cDNA purification step?**

In order to maximize yields, we recommend the following:

- a. Do NOT use cold water for the elution step. Use the D1 nuclease-free water included in the Ovation™ System kit at room temperature.
- b. Do NOT spin the columns at an incorrect speed. Strictly adhere to the guidelines in the User Guide.
- c. Use a fresh dilution of Ethanol from a fresh stock for any washing steps.
- d. Vortex the eluted cDNA sample prior to measuring the O.D.

**Q35. Should I purify the cDNA before determining 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.

**Q36. Do you recommend any RNA preparation methods?**

We do not specifically require one method of RNA preparation, as long as the method yields high quality, non-degraded RNA that is free of organic solvents and contaminants.

**Q37. What carrier RNAs do you recommend?**

We have tested and recommend the use of linear acrylamide as carrier (Ambion Cat. #9520).

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