

Frequently Asked Questions

FL-Ovation™ cDNA Biotin Module V2 (Cat # 4200-12)



Q1. What materials are provided with the FL-Ovation™ cDNA Biotin Module V2?

The Module provides all necessary buffers and enzymes for fragmentation and labeling of cDNA generated with a validated NuGEN Amplification System.

Q2. What equipment is required or will be useful?

Required equipment includes a microcentrifuge, pipettes, vortexer, a thermal cycler, and a U.V./Vis spectrophotometer. An Agilent Bioanalyzer or a similar instrument may be used for quality control.

Q3. What additional reagents are required for the FL-Ovation™ cDNA Biotin Module V2?

No additional reagents are required.

Q4. What type of cDNA should I use with the FL-Ovation™ cDNA Biotin Module V2?

You must use SPIA® cDNA generated with the WT-Ovation™ Pico RNA Amplification System (Cat.# 3300), the Ovation® RNA Amplification System V2 (Cat.# 3100), The Whole Blood Solution (Cat.# 3100 & 1300) or the WT-Ovation™ FFPE System V2 (Cat.# 3400). You can also use ST-cDNA generated by the WT-Ovation™ Exon Module (Cat # 2000).

Q5. How much labeled cDNA should I hybridize to a GeneChip® array?

We recommend using the entire 50 µl of the Fragmentation and labeling reaction for a standard GeneChip® array and 34 µl for a Midi format array hybridization, see Appendix A of product user guide.

Q6. Can I vary the amount of cDNA input to fragmentation and labeling?

The cDNA input amounts range from 3.75 to 5 µg, depending on your sample type and amplification kit used. Please see Table 2 in the Protocol section of the FL-Ovation™ cDNA Biotin Module V2 user guide. It is very important that the amount of cDNA input is kept consistent across all samples for each experiment.

Q7. Can I use any cDNA as starting material in the FL-Ovation™ cDNA Biotin Module V2?

No, the cDNA must be generated using a validated NuGEN Amplification System. Use of other cDNAs will result in poor performance.

Q8. How much fragmented and labeled cDNA yield can I expect?

Since this module does not require any purification, the total yield is equal to the input cDNA.

Q9. What is the size range of fragmented and labeled cDNA generated by the FL-Ovation™ cDNA Biotin Module V2?

As measured with an Agilent Bioanalyzer, 80% of product falls below 200 bases with an average peak at 85 bases.

Q10. Has NuGEN performed reproducibility studies on the FL-Ovation™ cDNA Biotin Module V2?

Yes, our studies have included sample to sample, lot-to-lot, and operator-to-operator reproducibility, see FL-Ovation™ cDNA Biotin Module V2 Technical Report #1 for some of these studies.

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Q11. Can the FL-Ovation™ cDNA Biotin Module V2 be used for fragmentation and labeling of RNA?

No.

Q12. Should I purify the cDNA before hybridization?

No. Purification of the fragmented and labeled product is not necessary.

Q13. What are the recommended storage conditions for the fragmented and labeled cDNA?

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

Q14. What types of arrays work with the FL-Ovation™ cDNA Biotin Module V2 cDNA?

The FL-Ovation™ cDNA Biotin Module V2 has been validated on Affymetrix 3' Expression and GeneChip® ST arrays.

Q15. Are the array hybridization reagents included in the FL-Ovation™ cDNA Biotin Module V2?

No. We only provide the reagents necessary for fragmentation and labeling of cDNA. We do provide a recommended procedure for hybridization, see Appendix A of product user guide.

Q16. What hybridization and wash protocols do you recommend for Affymetrix GeneChip® applications?

We recommend the same methods as the Affymetrix protocol with the following adjustments:

- a. Heat denature the hybridization cocktail at 99°C for 2 minutes
- b. Hybridize chips for 16-20 hours.
- c. Use the appropriate Affymetrix fluidics script, see Appendix A and B of product user guide.

Q17. What are the FL-Ovation™ cDNA Biotin Module V2 incubation temperatures for each step?

cDNA Fragmentation: 37 °C for 30 minutes, then 95 °C for 2 minutes, then cool to 4 °C

cDNA Labeling: 37 °C for 60 minutes, then 70 °C for 10 minutes, then cool to 4 °C.

Q18. Where can I safely stop in the fragmentation and labeling protocol?

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

Q19. How do I determine fragmentation success?

If you chose to determine the success of fragmentation, you may use the Agilent Bioanalyzer to inspect the size distribution of samples before and after fragmentation, see Appendix B of product user guide.

Q20. How should I qualify my cDNA for use with the FL-Ovation™ cDNA Biotin Module V2?

You must use cDNA generated with a validated NuGEN Amplification System product. The concentration of starting cDNA must be determined to ensure adequate input into the F&L reaction and therefore onto the arrays, please see Table 2 in the Protocol section of the FL-Ovation™ cDNA Biotin Module V2 user guide for cDNA input requirements. You may chose to further qualify the starting cDNA by performing qPCR assays as recommended in the appropriate NuGEN Amplification System user guides.

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