

Frequently Asked Questions

FL-Ovation™ cDNA Fluorescent Module

Cy™3 ULS™ Labeling (Cat # 4300)

Cy™3 & Cy™5 ULS™ Labeling (Cat # 4310)



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

The Module provides all necessary buffers, enzymes, dyes and purification columns for fragmentation and ULS-Cy™-dye 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 UV/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 Fluorescent Module?

No additional reagents are required for labeling and fragmentation. For hybridization, the additional reagents required are listed in the 'Additional Equipment' section of this user guide.

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

You may use SPIA™ cDNA generated with either the WT-Ovation™ FFPE (Cat.# 3400), WT-Ovation™ Pico (Cat.# 3300) or the Ovation™ RNA Amplification System V2 (Cat.# 3100).

Q5. Can I use any cDNA as starting material with this Module?

No, the cDNA must be generated using one of the NuGEN Amplification Systems listed above in Q4. Use of other cDNAs will result in poor performance.

Q6. How much labeled cDNA should I hybridize to an Agilent oligo array?

You should use 825 ng of Cy3 or 825 ng of the mixture of Cy3 and Cy5 labeled target per array for the Agilent Whole Human Genome 4x44K array.

Q7. Can I vary the amount of cDNA input to fragmentation and labeling?

We do not recommend modifying the amount of cDNA used in the labeling and fragmentation reaction as incomplete or excessive labeling and/or fragmentation may occur. We cannot guarantee success if you modify the recommended cDNA input.

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

Almost all the cDNA is recovered in the single column purification required after labeling, so the final yield is approximately equal to the input cDNA.

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

The fragmented cDNA will be approximately 100-200 bases in length. However, it is not recommended to run the Cy3 or Cy5 labeled material on the Bioanalyzer as the results will not be reflective of the actual size due to the presence of the Cy dye molecules.

Q10. Has NuGEN performed reproducibility studies on this Module?

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

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Q11. Can the FL-Ovation™ cDNA Fluorescent Module be used for fragmentation and labeling of RNA?
No. This product does not work for RNA fragmentation.

Q12. Should I purify the cDNA before hybridization?

It is not necessary to purify the labeled and fragmented cDNA beyond what is described in the User Guide.

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 and prolonged exposure to light.

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

The FL-Ovation™ cDNA Fluorescent Module has been validated using the Agilent Whole Human Genome 4X44K oligo microarrays. It is likely that other Agilent Dual-mode gene expression DNA oligo microarrays will work, but this has not been tested. The use of platforms other than Agilent expression arrays for this product is not supported by NuGEN.

Q15. Are the array hybridization reagents included in the module.

The only hybridization reagent provided with this kit is a special Blocking Reagent needed. Other hybridization reagents required are the Agilent 2X Hybridization Buffer and the 10X Blocking Agent. For part numbers and order information, refer to the appropriate Agilent microarray user guide.

Q16. What hybridization and wash protocols do you recommend for Agilent oligo microarrays?

Follow the standard protocols as recommended by the array manufacturer, except hybridize for 40 hours.

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

cDNA Labeling: 85 °C for 15 minutes, then 4°C,
cDNA Fragmentation: 37 °C for 30 minutes, then 4°C

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

You may safely stop after cDNA labeling and store the labeled cDNA at -20°C.

Q19. How should I qualify my cDNA for use with the FL-Ovation™ cDNA Fluorescent Module?

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 labeling and fragmentation reactions and therefore onto the arrays. You may choose to further qualify the starting cDNA by performing qPCR assays as recommended in the appropriate NuGEN Amplification System user guide(s).

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