

Frequently Asked Questions

WT-Ovation™ Exon Module

(Cat # 2000-12)



Q1. What materials are provided with the WT-Ovation™ Exon Module?

The Module provides all necessary buffers and enzymes for converting cDNA generated with a validated NuGEN Amplification System into sense target cDNA (ST-cDNA).

Q2. What equipment is required or will be useful?

Required equipment include a microcentrifuge, pipettes, vortexer, 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 WT-Ovation™ Exon Module?

Zymo Clean & Concentrator™-25 purification columns.

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

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

Q6. Can I vary the amount of cDNA input to ST-cDNA Generation?

For RNA samples of good quality, we recommend 3 µg input for cDNA generated with either WT-Ovation™ FFPE System or the WT-Ovation™ Pico System. For RNA from FFPE or degraded RNA sources, we recommend 4 µg cDNA input. It is 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 WT-Ovation™ Exon Module?

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

Q8. How much ST-cDNA yield can I expect?

6-8 µg

Q9. What is the size range of ST-cDNA generated by the WT-Ovation™ Exon Module?

As measured with an Agilent Bioanalyzer, the ST-cDNA is shorter than the template cDNA.

Q10. Has NuGEN performed reproducibility studies on the WT-Ovation™ Exon Module?

Yes, our studies have included sample to sample, lot-to-lot, and operator-to-operator reproducibility. See WT-Ovation™ Exon Module Technical Report #1 for some of these studies.

Q12. Should I purify the input cDNA before ST-cDNA generation?

Yes. The protocol requires a specific quantity of amplified cDNA and the cDNA must be purified in order to be accurately quantitated.

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Q13. What are the recommended storage conditions for the ST-cDNA?

The ST-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 WT-Ovation™ Exon Module cDNA?

The WT-Ovation™ Exon Module has been validated to generate cDNA targets ready for fragmentation and labeling with the FL-Ovation cDNA Biotin Module V2 prior to hybridization on Affymetrix ST arrays.

Q15. Are the fragmentation and labeling reagents included in the WT-Ovation™ Exon Module?

No. This kit only includes the reagents necessary for generating ST-cDNA. The fragmentation and labeling components are included in the FL-Ovation™ cDNA Biotin Module V2.

Q15. Are the array hybridization reagents included in the WT-Ovation™ Exon Module?

No. This kit only includes the reagents necessary for generating ST-cDNA.

Q16. What are the WT-Ovation™ Exon Module incubation temperatures for each step?

Primer Anneal: 95 °C for 5 minutes, then cool to 4 °C

ST-cDNA Generation: 4 °C for 1 minute, 30 °C for 10 minutes, 42 °C for 60 minutes, 70 °C for 10 minutes, then 4 °C forever.

Q17. Where can I safely stop in the ST-cDNA generation protocol?

You may stop after the second incubation step (ST-cDNA generation) just before ST-cDNA purification.

Q20. How should I qualify my cDNA for use with the WT-Ovation™ Exon 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 ST-cDNA reaction. 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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