

NOD2 R702W ToolSet™ for LightCycler™

Lyophilized ToolSet for PCR using the LightCycler™ Instrument. Licensed by Roche Diagnostics GmbH

Order#: NOD2 702 - 16

1 ToolSet for 16 reactions

Store at 4°C, protected from light.
Exposure to light may especially damage
the OligoTool™ tube (vial with red cap).

For use with LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102)

1. ToolSet contents

| Vial | Label | Content | Quantity |
|---------------------|------------------|--|---|
| | | | NOD2 702 - 16 |
| 1, Red cap | OligoTool | - lyophilized oligos for PCR - contains mutation detection and anchor probe, primers | For 16 tests Dissolved: 50 µL |
| 2, Green cap | Control | - lyophilized heterozygous DNA | Dissolved: 20 µL |
| 3, Blue cap | Solvent | - to dissolve OligoTool / Control | 1000 µL of Solvent |

Additional equipment and reagents required but not supplied :
LightCycler-DNA Master Hybridization Probes, 10 x conc.Cat.No.: 2 015 102, including 25mM MgCl₂;
LightCycler instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description The ToolSet is specifically adapted for genotyping NOD2 for the Amino Acid exchange R702W, corresponding to the C2104T mutation at the nucleotide level, by LightCycler PCR with Melting Curve Analysis. Fluorescent detection and anchor probes and the primer pair have been optimized for specific amplification of targets and optimal genotype discrimination.

Control material Heterozygous control DNA, lyophilized.

Storage of ToolSet and Solutions Store at +4°C when lyophilized, protected from light. The unopened lyophilized ToolSet is stable at +4°C for 12 months from date of manufacture if protected from light. When dissolved store at +4°C for a maximum of 4 weeks, or at -20°C for longer periods (months), protected from light. Avoid freezing and thawing.

3. Preparation for LightCycler PCR

Toolset preparation Dissolve the content of the OligoTool tube (Red Cap) with 50 µl of Solvent.
Dissolve the content of the Control tube (Green Cap) with 20 µl of Solvent.

1. Before opening tubes, centrifuge them quickly.
2. Add Solvent into OligoTool tube and Control tube as above.
3. Recap tubes, vortex gently.
4. Before opening tubes, centrifuge them quickly.
5. Proceed to Reaction Mix preparation.

Primers ? You don't have to add primers.

Probes ? You don't have to add probes.

Reaction Mix Preparation For 1 (One) reaction, prepare the Reaction Mix as shown in the following table :

| Reagent | µL |
|------------------------------------|--------------------|
| OligoTool NOD2 702 -16 dissolved | 2.8 |
| Solvent NOD2 702 -16 | 9.6 |
| MgCl ₂ 25 mM | 1.6 (final 3.0 mM) |
| Master Hybridization Probes 10x | 2 |
| Total Reaction Mix | 16 |
| + Your DNA or Control NOD2 702 -16 | 4 |
| Grand Total | 20 |

Use Master Hybridization Probes 10x and MgCl₂ 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc.

(Roche Cat.No.: 2 015 102, including 25mM MgCl₂).

For multiple reactions, multiply the indicated volumes appropriately.

Positive Control Always run a positive control with the samples.
Use the dissolved heterozygous Control NOD2 702 -16 DNA (Green Cap).

Negative control Always run a negative control with the samples. To prepare a negative control, replace the template DNA with Solvent (Blue Cap).

Extraction of genomic DNA You can use different Kits for DNA isolation, either with a manual method or with an automated system. The elution buffers should be salt-free. Example : Roche High Pure PCR Template Preparation Kit (Cat.No. 1 796 828)

Application The NOD2 R702W ToolSet™ for LightCycler™ allows the detection of the single nucleotide mutation C2104T resulting in the NOD2 R702W variant of the NOD2 gene which impairs LPS-induced signalling and is associated with diminished responsiveness to bacterial LPS in NOD2 – expressing cells and is found at increased frequencies in samples from Crohn's disease.

Note : This ToolSet was developed for use in life science research only.

Note : This ToolSet uses the same Time-Temperature-Protocol as the **NOD2 G908R** and **NOD2 1007 fs** ToolSets™ for LightCycler™ and can be used in the same LC run.

4. LightCycler Settings and Experimental Protocol

Denaturation

| Cycle Program Data | Value |
|-----------------------------------|------------------|
| Cycles | 1 |
| Analysis Mode | None |
| Temperature Targets | Segment 1 |
| Target Temperature (°C) | 95 |
| Incubation time (s) | 120 |
| Temperature Transition Rate (°/s) | 20.0 |
| Secondary Target Temperature (°C) | 0 |
| Step Size (°C) | 0 |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

Amplification

| Cycle Program Data | Value | | |
|-----------------------------------|------------------|------------------|------------------|
| Cycles | 40 | | |
| Analysis Mode | None | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 3 |
| Target Temperature (°C) | 95 | 54 | 72 |
| Incubation time (s) | 1 | 10 | 10 |
| Temperature Transition Rate (°/s) | 20.0 | 20.0 | 5.0 |
| Secondary Target Temperature (°C) | 0 | 0 | 0 |
| Step Size (°C) | 0 | 0 | 0 |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | Single | None |

Melting Curve Analysis

| Cycle Program Data | Value | | |
|-----------------------------------|------------------|------------------|------------------|
| Cycles | 1 | | |
| Analysis Mode | Melting Curves | | |
| Temperature Targets | Segment 1 | Segment 2 | Segment 3 |
| Target Temperature (°C) | 95 | 40 | 85 |
| Incubation time (s) | 60 | 120 | 0 |
| Temperature Transition Rate (°/s) | 20.0 | 20.0 | 0.1 |
| Secondary Target Temperature (°C) | 0 | 0 | 0 |
| Step Size (°C) | 0 | 0 | 0 |
| Step Delay (Cycles) | 0 | 0 | 0 |
| Acquisition Mode | None | None | Continuous |

Cooling

| Cycle Program Data | Value |
|-----------------------------------|------------------|
| Cycles | 1 |
| Analysis Mode | None |
| Temperature Targets | Segment 1 |
| Target Temperature (°C) | 40 |
| Incubation time (s) | 30 |
| Temperature Transition Rate (°/s) | 20.0 |
| Secondary Target Temperature (°C) | 0 |
| Step Size (°C) | 0 |
| Step Delay (Cycles) | 0 |
| Acquisition Mode | None |

Fluorescence display mode

With LC program 3.3 use F2/F1 or preferably F2 with colour compensation; gains: F1=1; F2=15.
 With LC program 3.5 use automatic gain control, preferably with colour compensation.

5. Typical results

Introduction

Use the Melting Curve program to genotype the human genomic DNA research samples. The melting peaks allow discrimination between the homozygous (wild type or mutant) and the heterozygous samples. Figure 1 shows a typical result obtained with the NOD2 R702W ToolSet™ for LightCycler™ :

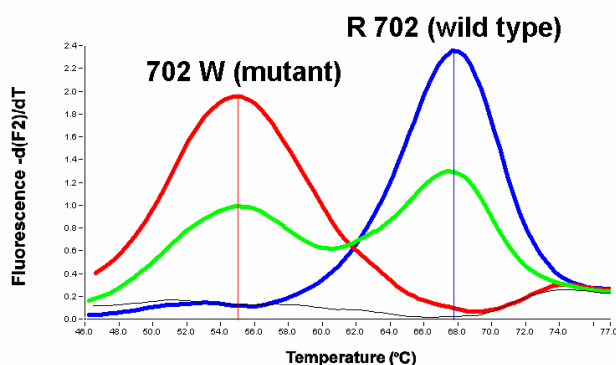


Figure 1 : Melting curve analysis of possible genotypes of the NOD2 sequence at AA 702.

BLUE : Homozygote Wild Type R702, **RED** : Homozygote Mutant 702W, **BLACK** : No DNA Control
GREEN : The Heterozygote R702W Control contained in the ToolSet, Control NOD2 702-16.

Conditions : Color compensation and Digital Filter enabled,
Calculation Method : Polynomial, Degrees to Average : 12.0
Red Cursor : $T_m = 55.0^\circ\text{C}$, Blue Cursor : $T_m = 67.7^\circ\text{C}$

Note : The values for the respective melting temperatures may vary for $\pm 2.5^\circ\text{C}$ between different experiments. The Delta T between the melting peaks for different genotypes may vary $\pm 1.0^\circ\text{C}$. The NOD2 R702W ToolSet™ has been developed for and validated with the LightCycler™ and its original accessory materials and reagents. Performance of the ToolSet with other instruments, accessories and reagents has not been validated by ratiogen.

7. Notices to Purchaser : Licenses and Trademarks, Prohibition of Resale

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