GHR fl - d3 ToolSet™ for LightCycler™

(Full Length/Deletion of Exon 3 – Variants of the Growth Hormone Receptor)

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

Order#: GHR fl-d3

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
			GHR fl-d3
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 $MgCl_2$;
LightCycler instrument, LightCycler capillaries, DNA extraction materials

2. Introduction

2.1. Product overview

ToolSet description

This ToolSet is specifically designed for genotyping the Human Growth Hormone receptor (GHR) for presence of the full length allele (GHRfl) or deletion of exon 3 (GHRd3) by LightCycler PCR with Melting Curve Analysis. Primer pair and fluorescent detection / anchor probes have been optimized for specific amplification of a 169 bp segment of either repeat **1** (5' of exon 3) in case of GHRfl or repeat **2** (3'of exon 3) in case of GHRd3. Repeats 1 and 2 are identical except for 3 SNP's. The SNP C14G of the repeat is typed by melting curve analysis and thus allows discrimination of the GHRfl (C) from the GHRd3 (G) allele (Pantel J. JBC 2000;275(25):18664-9, PubMed ID 10764769).

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 ?
Probes ?

You don't have to add primers.
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 GHR fl-d3, dissolved	2.8
Solvent GHR fl-d3	9.6
$MgCl_2$ 25 mM	1.6 (final 3 mM)
Master Hybridization Probes 10x	2
Total Reaction Mix	16
+ Your DNA or Control GHR fl-d3	4
Grand Total	20

Use Master Hybridization Probes 10x and $MgCl_2$ 25 mM from Roche LightCycler-DNA Master Hybridization Probes, 10 x conc. (Roche Cat.No.: 2 015 102, including 25mM $MgCl_2$).
For multiple reactions, multiply the indicated volumes appropriately.

Positive Control

Always run a positive control with the samples. Use the dissolved heterozygous Control GHR fl-d3 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 **GHR fl-d3 ToolSet™ for LightCycler™** has been specifically designed for the detection of the frequent **Full length – Deletion of Exon 3 (fl-d3)** polymorphism in the Human Growth Hormone Receptor (GHR) gene.
About **40 % of Caucasians carry the d3 Allele** which has been associated with **enhanced growth in children treated with human growth hormone** (Dos Santos C., Nat Genet. 2004; 36(7) : 720-4, PubMed ID 15208626). The **fl-d3 polymorphism** has also been associated with **body composition** (BMI, fat mass, waist circumference) and **metabolic risk factors** (fasting insulin, uric acid, HDL, triglycerides, apolipoprotein B, leptin and diastolic blood pressure) (Kratzsch J. Clin Endocrinol 2001; 54(1) : 61-8, PubMed ID 11167927 and Seidel B. Eur J Endocrinol. 2003; 148(3) : 317-24, PubMed ID 12611612) and may thus be involved in the **pathogenesis of obesity, diabetes and atherosclerosis.**

More information is available in the *Genes* section at www.Genes-4U.com

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

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)	60
Temperature Transition Rate (°/s)	20
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

Amplification

Cycle Program Data	Value		
Cycles	**60**		
Analysis Mode	None		
Temperature Targets	**Segment 1**	**Segment 2**	**Segment 3**
Target Temperature (°C)	95	55	72
Incubation time (s)	5	**10**	**10**
Temperature Transition Rate (°/s)	20	**5**	**5**
Secondary Target Temperature (°C)	0	0	0
Step Size (°C)	0	0	0
Step Delay (Cycles)	0	0	0
Acquisition Mode	None	Single	None

Note ! Homozygous fl/fl samples show minimal or no amplification signal since here the Tm of hybridisation probes is below the PCR annealing temperature. This feature has no influence on melting curve analysis.

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	80
Incubation time (s)	60	60	0
Temperature Transition Rate (°/s)	20	20	**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
Secondary Target Temperature (°C)	0
Step Size (°C)	0
Step Delay (Cycles)	0
Acquisition Mode	None

LC Program Version and Fluorescence Display Mode

Developed with LC Program Version 3.5. Use F2/F1 or preferably F2 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 of Full Length and Exon 3 – Deletion genotypes of the human Growth Hormone Receptor. Figure 1 shows a typical result obtained with the GHR fl-d3 ToolSet™ for LightCycler™ :

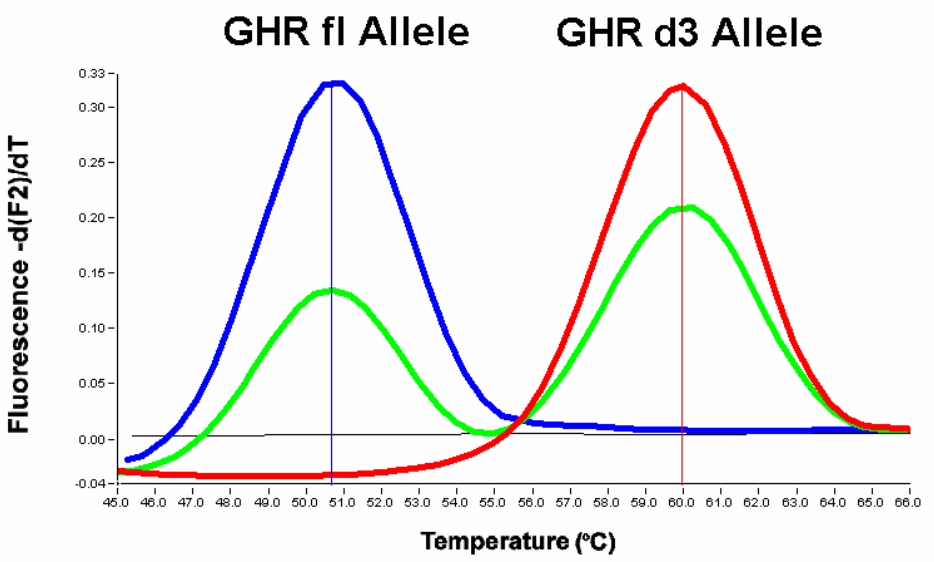

Figure 1 : Melting curve analysis of possible genotypes of the fl-d3 polymorphism in the GHR gene.

BLUE : Homozygote for **GHR fl/fl (wild type)** , **RED** : Homozygote for **GHR d3/d3**, **BLACK** : No DNA Control.
GREEN : Heterozygote Control contained in the ToolSet, Control **GHR fl/d3 HET**.
Blue Cursor : T_m = 50.7 ^{0}C **, Red Cursor** : T_m = 60.0 ^{0}C.

Conditions : LC program version 3.5 with automatic gain setting, No Color compensation,
Digital Filter enabled, Calculation Method : Polynomial, Degrees to average : 10.

Note : The values for the respective melting temperatures may vary for +/- 2.5 ^{0}C between different experiments. The Delta T between the melting peaks for different genotypes may vary +/- 0.5 ^{0}C.
The GHR fl-d3 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 Genes-4U.

7. Notices to Purchaser

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