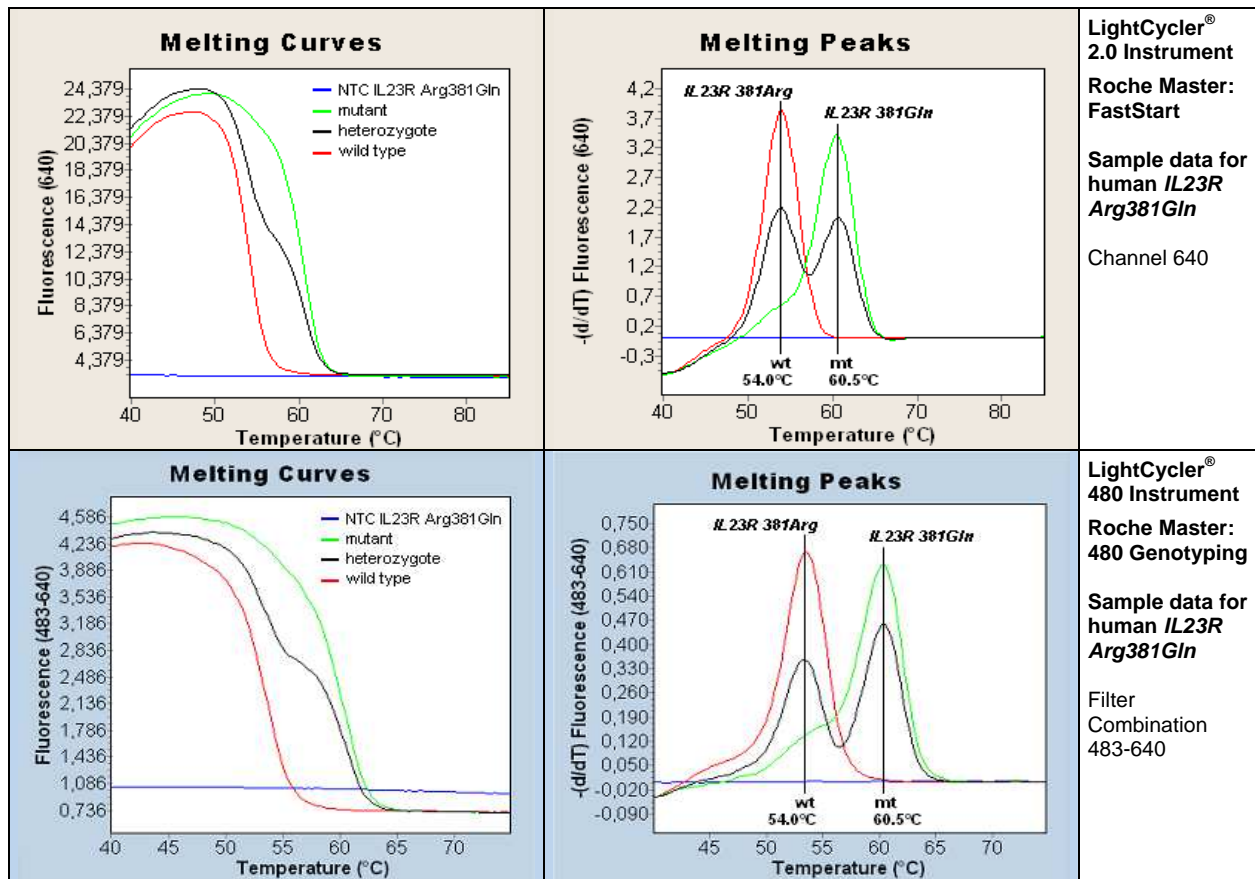


## 8. Sample data - typical results



**Fig.1. Sample data for the human *IL23R Arg381Gln* detection system.**

**Upper panels:** Data from LightCycler® 2.0 Instrument. Left panel channel 640 melting curves for human *IL23R Arg381Gln*. Right panel channel 640 melting peaks for human *IL23R Arg381Gln*.

**Lower panels:** Data from LightCycler® 480 Instrument. Left panel filter comb. 483-640 melting curves for human *IL23R Arg381Gln*. Right panel filter comb. 483-640 melting peaks for human *IL23R Arg381Gln*.

Genotype:	wild type homozygote <i>381Arg</i>	heterozygote <i>381Arg/Gln</i>	mutant homozygote <i>381Gln</i>
Number of melting peaks (color)	1 (red)	2 (black)	1 (green)
Melting temperature of peaks	54.0°C	54.0°C and 60.5 °C	60.5°C
Temperature difference between peaks	---	6.5°C	---
Phenotype	no effect	lower risk for inflammatory diseases	lower risk for inflammatory diseases

**Tab. 3. Typical analysis results (LightCycler® 2.0 Instrument, Roche Master: FastStart)**

**Note:** The values of the respective melting temperatures ( $T_m$ ) may vary  $\pm 2.5^\circ\text{C}$  between different experiments.

The  $\Delta T$  between the melting peaks for heterozygote genotypes may vary  $\pm 1.5^\circ\text{C}$ .

Samples with deviating melting curves should be subject to further investigations; sequence analysis can be provided by TIB MOLBIOL Berlin (contact [service@tib-molbiol.de](mailto:service@tib-molbiol.de)).

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These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany. LightCycler® hybridization probes produced under license from Roche Diagnostics GmbH.



## LightMix<sup>®</sup> Kit human *IL23R Arg381Gln*

Cat.-No. 40-0349-16

Version 05/2011: double amount of control DNA per vial

Kit with reagents for the detection of the human *IL23R Arg381Gln* DNA polymorphism using the LightCycler<sup>®</sup> 1.x / 2.0 / 480 Instruments.

Lyophilized mix of primers and probes (6 tubes with 16 rxns each) for a total of 96 reactions with a final volume of 20 µl each. **Store protected from light at room temperature (18-25°C), do NOT freeze!**

### Additional reagents required

#### Roche Diagnostics:

LightCycler<sup>®</sup> FastStart DNA Master<sup>PLUS</sup> HybProbe Cat.-No. 03 515 575 001

or LightCycler<sup>®</sup> FastStart DNA Master HybProbe Cat.-No. 03 003 248 001

or LightCycler<sup>®</sup> 480 Genotyping Master (LightCycler<sup>®</sup> 480 Instrument only) Cat.-No. 04 707 524 001

HighPure PCR Template Preparation Kit Cat.-No. 11 796 828 001

## 1. Introduction

IL23R (Interleukin 23 receptor, OMIM\*607562) is one subunit of the receptor for IL23A/IL23 and is thus involved in IL23 signalling. The protein is associated with JAK2. The IL23R variant Arg381 to Gln (rs11209026), resulting from the G to A substitution at nucleotide 1142, shows a strong protection against Crohn's disease, inflammatory bowel disease and other inflammatory diseases<sup>1,2,3</sup>. The IL-23R genotype of bone marrow donors is associated with a reduced risk of acute graft-versus-host disease (GVHD); Elmaagacli et al. reported only 4% instead of 23% severe cases (P=0.003), and also less deaths due to remission<sup>4</sup>. The allele frequency for the Caucasian population is about 3 - 10%.

The LightMix<sup>®</sup> Kit human *IL23R Arg381Gln* provides a fast, easy and accurate system to identify the genotype of this target in a nucleic acid extract.

This LightMix<sup>®</sup> Kit is tested with the Roche Diagnostics 'LightCycler<sup>®</sup> FastStart DNA Master Hybridization Probe' and the Roche Diagnostics 'LightCycler<sup>®</sup> FastStart<sup>PLUS</sup> DNA Master Hybridization Probe' in the LightCycler<sup>®</sup> 2.0 Instrument and with the Roche Diagnostics 'LightCycler<sup>®</sup> FastStart DNA Master Hybridization Probe' and the Roche Diagnostics 'LightCycler<sup>®</sup> 480 Genotyping Master' in the LightCycler<sup>®</sup> 480 Instrument (384 and 96 well format).

<sup>1</sup>A Genome-Wide Association Study Identifies IL23R as an Inflammatory Bowel Disease Gene. Dürr H.R. et al. *Science* **314**: 1461-1463 (2006).

<sup>2</sup>IL23R Arg381Gln is associated with childhood onset inflammatory bowel disease in Scotland. Van Limbergen J. et al. *Gut* **56**:1173-1174 (2007).

<sup>3</sup>Detailed genetic characterization of the psoriasis-associated gene IL23R. Schrodi S.J. et al. Poster (2007).

<sup>4</sup>Relation of an interleukin-23 receptor gene polymorphism to graft-versus-host disease after hematopoietic-cell transplantation. Elmaagacli AH, Koldehoff M, Landt O, Beelen DW. *Bone Marrow Transplant* advance online publication 10.1038/sj.bmt.1705980 (2008).

## 2. Description

A 287 bp fragment of the human *IL23R* gene is amplified with specific primers. The resulting PCR fragments are analyzed with hybridization probes labeled with LightCycler<sup>®</sup> Red 640 (detected in channel 640). The genotype is identified by running a melting curve with specific melting points (T<sub>m</sub>). The wildtype *IL23R 381Arg* exhibits a T<sub>m</sub> of 54.0°C in channel 640. The mutant *IL23R 381Gln* exhibits a T<sub>m</sub> of 60.5°C in channel 640.

The supplied control DNA allows for the accurate comparison with unknown samples.

For use in LightCycler<sup>®</sup> 1.x Instruments with software version 3.5.3 read channel F2 instead of channel 640, channel F3 instead of channel 705 and channel F1 instead of channel 530 for detection. We recommend upgrading LightCycler<sup>®</sup> 1.x Instruments to software version 4.1.

For use in LightCycler<sup>®</sup> 480 Instruments use filter combination 483-640 instead of channel 640.

### 3. Set contents

- 6 Vials with red caps containing premixed lyophilized primers and probes for 16 PCR reactions each of *IL23R Arg381Gln*
- 3 Vials with colorless caps containing control DNA (*IL23R Arg381Gln*: wt, hetero, mt), 10<sup>5</sup> target equivalents per reaction

### 4. Programming

The protocol consists of four program steps

- 1: Denaturation: sample denaturation and enzyme activation
- 2: Cycling: PCR-amplification of the target DNA
- 3: Melting: melting curve analysis for identification of the PCR product derived from the target DNA
- 4: Cooling: cooling the instrument

Program Step:		Denaturation	Cycling			Melting			Cooling
Settings LC 1.x/2.0	<b>Parameter</b>								
	Analysis Mode	None	Quantification mode			Melting Curves mode			None
	Cycles	1	55			1			1
	Segment	1	1	2	3	1	2	3	1
	Target [°C]	95	95	60	72	95	40	85	40
	Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:15	00:00:20	00:00:20	00:00:00	00:00:30
	Ramp Rate [°C/s]	20	20	20	20	20	20	0.2	20
	Acquisition Mode	None	None	Single	None	None	None	Continuous	None
Settings LC 480	Analysis Mode	None	Quantification mode			Melting Curves mode			None
	Cycles	1	55			1			1
	Segment	1	1	2	3	1	2	3	1
	Target [°C]	95	95	60	72	95	40	75	40
	Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:15	00:00:30	00:02:00	00:00:00	00:00:30
	Ramp Rate [°C/s] <b>96</b>	4.4	4.4	2.2	4.4	4.4	1.5	-	1.5
	Ramp Rate [°C/s] <b>384</b>	4.6	4.6	2.4	4.6	4.6	2.0	-	2.0
	Acquisitions [per °C]	-	-	-	-	-	-	3	-
Acquisition Mode	None	None	Single	None	None	None	Continuous	None	

### 5. Data analysis

Perform data analysis, as described in the LightCycler® Instrument operator's manual. We recommend using the Polynomial Calculation Method for analyzing the melting peaks. The melting temperature is determined with the manual T<sub>m</sub> setting.

View human *IL23R Arg381Gln* data in channel 640, "T<sub>m</sub> Calling" Analysis mode (LightCycler® 2.0 / 480 Instruments) or Melting Curves mode (LightCycler® 1.x Instrument). The negative control (NTC) should show no signal.

### 6. Product characteristics

PCR results are obtained within 1 hour.

#### Sensitivity

These reagents detect 1 ng of genomic DNA.

#### Measuring range

The measuring range of the assay is 1 ng to 100 ng of genomic DNA.

#### Storage and Stability

- Lyophilized reagents are stable for at least 3 months after shipment if stored protected from light at room temperature (18-25°C).
- **Do not freeze** lyophilized reagents.
- Dissolved reagents are stable for at least 5 days if stored protected from light and refrigerated (4°C).

## 7. Experimental protocol

The following procedure was developed for use with the LightCycler® 1.x / 2.0 / 480 Instruments. Start programming before preparing the solutions. See the LightCycler® Instrument operator's manual for details.

**Sample material:** Use aqueous nucleic acid preparations (e.g. Roche Diagnostics 'High Pure PCR Template Preparation Kit').

**Negative control:** Always run at least one no-template control (NTC) - replace the template DNA with water.

**Positive control:** Run a positive control - replace the template DNA with the provided control DNA.

### 7.1. Preparation of parameter-specific reagents (16 reactions):

One reagent vial with a **red** cap contains all primers and probes to run 16 LightCycler® reactions for human *IL23R Arg381Gln*.

Add 66 µl PCR-grade water to each reagent vial, mix the solution (vortex) and spin down.

► Use 4 µl **reagent** for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C. Avoid prolonged exposure to light.

### 7.2. Preparation of the control DNA

Add 80 µl PCR-grade water to each vial ( $1.6 \times 10^6$  target molecules) with a colorless cap. Mix the target DNA by pipetting the solution up and down 10 times (final concentration:  $10^5$  target molecules in 5 µl).

► Use 5 µl control DNA for a 20 µl PCR reaction.

| This solution is stable at least five days when stored refrigerated at 4°C, for long term storage freeze at -20°C, avoid repeated freezing thawing cycles. For the heterozygote control DNA provided with the kit please note that the relative amounts of wild type DNA and mutant DNA may change during time.  
Please note that opening of these vials may cause contaminations of the work-space (aerosol).

### 7.3. Preparation of the LightCycler® reaction mix

In a cooled reaction tube, prepare the reaction mix by multiplying each volume for a single reaction by the number of reactions to be cycled plus one additional reaction.

For use with the Roche Master:	FastStart	FastStart <sup>PLUS</sup>	480 Genotyping
<b>Component</b>	<b>Single reaction</b>		
water, PCR-grade (colorless cap, provided with the Roche Master kit)	7.4 µl	7.0 µl	7.0 µl
Mg <sup>2+</sup> solution 25 mM (blue cap, provided with the Roche FastStart kit)	1.6 µl	--	--
<b>reagent</b> mix (parameter specific reagents containing primers and probes, see 7.1.)	4.0 µl	4.0 µl	4.0 µl
Roche Master (red/yellow cap, for preparation see Roche manual)	2.0 µl	4.0 µl	4.0 µl
<b>Volume of reaction mix</b>	<b>15.0 µl</b>	<b>15.0 µl</b>	<b>15.0 µl</b>

Mix gently, spin down and transfer 15 µl each of the reaction mix to a LightCycler® capillary (LightCycler® 1.x / 2.0 Instrument) or to a multiwell plate (LightCycler® 480 Instrument).

Add 5 µl of sample or control DNA to each capillary or well for a final reaction volume of **20 µl**.

Start run.