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## LightMix<sup>®</sup> Kit *JAK2* Exon 12 Mutation Screening

Cat.-No. 40-0356-16

Control DNA for E543-D544del included

Kit with reagents for the detection of *JAK2* exon 12 mutations in codons 537-544 in genomic DNA using the LightCycler<sup>®</sup> 2.0 instrument. This kit does not detect the V617F mutation located in exon 14.

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!**

### 1. Introduction

Besides Chronic Myeloid Leukaemia (CML) there are a few other important chronic myeloproliferative diseases (CMPD), namely Polycythaemia Vera (PV, exceeded proliferation of red blood cells), Essential Thrombocythaemia (ET) and chronic idiopathic myelofibrosis. The acquired *Janus kinase 2 (JAK2) V617F* point mutation can be found in more than 90% of patients with PV and in 50% of patients with other CMPDs.<sup>1,2,3</sup> The *JAK2 V617F* mutation is covered by patents from Ipsogen.

Additional mutations have been described for the codons 537-544 in exon 12, in particular Lys539Leu, His538Gln-Lys539Leu, del537-538insLeu, Glu543-Asp544del and Asn542-Glu543del.<sup>4</sup>

This LightMix<sup>®</sup> three-channel mutation screening kit is designed to detect these variants in genomic and cDNA samples. The kit has been tested with the Roche Diagnostics 'LightCycler<sup>®</sup> FastStart DNA Master Hybridization Probes' in the LightCycler<sup>®</sup> 2.0 instrument. This kit can not be used with other Roche Diagnostics Real-Time-PCR instruments.

<sup>1</sup>Acquired mutation of the tyrosine kinase *JAK2* in human myeloproliferative disorders. Baxter et al., *Lancet* 2005; 365: 1054

<sup>2</sup>Validation of two clinically useful assays for evaluation of *JAK2 V617F* mutation in chronic myeloproliferative disorders. McClure R, Mai M, Lasho T. *Leukemia*. 2006 Jan;20(1):168-71

<sup>3</sup>Detection of the *JAK2(V617F)* mutation in myeloproliferative disorders by melting curve analysis using the LightCycler system. Olsen et al., *Arch Pathol Lab Med*. 2006; 130:997-1003

<sup>4</sup>*JAK2* exon 12 mutations in polycythemia vera and idiopathic erythrocytosis. Scott et al., *N Engl J Med*. 2007; 356: 459-68

### 2. Description

A 267 bp long fragment from *JAK2* exon 12 is amplified with primers; the amplification curve does not provide any diagnostic information but gives an estimate on the amount of target. Three different labelled sensor probes specific for mutation 539Leu (LC640), for N542-E543del (LC610) and for del537/539insL (LC670) bind to the fragment and allow to analyze the amplified sequence for these mutations and other variations, running a melting curve analysis.

The use of a color compensation file generated with the LightMix<sup>®</sup> Color Compensation HybProbe 530/640/690 kit (order no. 40-0318-00) is a prerequisite to detect the internal control.

The supplied control DNA allows comparison with unknown genomic samples to be analyzed.

### 3. Set Contents

- 6 Vials with **red** cap containing premixed lyophilized primers and probes for 16 PCR reactions
- 6 Vials with **colorless** cap containing each 10<sup>5</sup> target equivalents per reaction control DNA:  
*JAK2 wild type, JAK2 539Leu, 538Q-539L, del537-539inL, E543-D544del and N542-E543del.*

### 4. Additional Reagents and Items Required

ColorCompensation HybProbe order n° 40-0318-00

LightCycler<sup>®</sup> FastStart DNA Master Hybridization Probes

HighPure PCR Template Preparation Kit

Roche Diagnostics

Cat.-No. 05 997 704 001

Cat.-No. 03 003 248 001

Cat.-No. 11 796 828 001

## 5. Product Characteristics

PCR results are obtained within 1 hour.

### Sensitivity

These reagents detect 5 ng of genomic DNA (in an exemplary system, using cloned targets).

### Measuring range

The measuring range of the assay is 5 ng to 100 ng of *JAK2* genomic DNA.

### Storage and Stability

- Lyophilized reagents stable for 12 months after production. See expiry date on outer product label.
- Store protected from light at 4°C to 25°C. **Do not freeze** lyophilized reagents.
- Dissolved reagents stable for at least 10 days when stored protected from light and cooled (4°C to 10°C).
- Dissolved reagents can be long-term stored frozen at -15 to -25°C. Minimize multiple thaw-freeze cycles.

## 6. Contents, Material Safety Data, Certificate of Origin

Product contains: 99.8% Synthetic oligonucleotides (< 100 microgramm)  
0.1% CAS 77-86-1 Tris (hydroxymethyl) aminomethane  
0.1% CAS 60-00-4 Ethylenediamine tetracetic acid (EDTA)

Product not hazardous, not toxic, not IATA-restricted. HS code 29349990.

According to OSHA 29CFR1910.1200, Australia [NOHSC:1005, 1008 (1999)] and the EU Directives 67/548/EC and 1999/45/EC any products which not contain more than 1% of a component classified as hazardous or classified as carcinogenic do not require a Material Safety Data Sheet (MSDS).

Product is not from human, animal or plant origin. Country of Origin: Germany

## 7. 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:	Denaturation	Cycling			Melting			Cooling
<b>Parameter</b>								
Analysis Mode	None	Quantification mode			Melting Curves mode			None
Cycles	1	40			1			1
Segment	1	1	2	3	1	2	3	1
Target [°C]	95	95	53	72	95	40	85	40
Hold [hh:mm:ss]	00:10:00	00:00:10	00:00:10	00:00:20	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	Continu.	None

## 8. Data Analysis

Perform data analysis, as described in the LightCycler® Instrument operator's manual.

Switch the color compensation mode on. If this mode is not enabled run the color compensation program. Follow the instructions in the manual Color Compensation Kit HybProbe 530/640/690.

View *JAK2* genomic data in channel 610, 640 and 670 "Tm Calling" Analysis mode (LightCycler® 2.0 Instrument). The negative control (NTC) must show no signal.

## 9. Experimental Protocol

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

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

**Negative control:** Always run at least one negative control - replace the template DNA with water.

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

### 9.1. Preparation of Parameter-Specific Reagents (16 reactions):

One reagent vial with a **red** cap contains primers and probes to run 16 reactions.

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.

### 9.2. Preparation of the Control DNA

Add 40 µl PCR-grade water to each vial ( $8 \times 10^5$  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 mixed 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 reopening of these vials may cause contaminations of the work-space (aerosol).

### 9.3. Preparation of the 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 FastStart Master	
Component	Single reaction
water, PCR-grade (colorless cap, provided with the Roche Master kit)	7.4 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 9.1.)	4.0 µl
Roche Master (red cap, for preparation see Roche manual)	2.0 µl
<b>Volume of reaction mix</b>	<b>15.0 µl</b>

Mix gently, spin down and **transfer 15 µl** of the reaction mix to a capillary or well.

**Add 5 µl** of sample or standard to each capillary or well for a final reaction volume of 20 µl. Close the capillaries / attach a foil to the multiwell plate and seal, and spin down.

**Start run.**

## 10. Sample Data - Typical Results

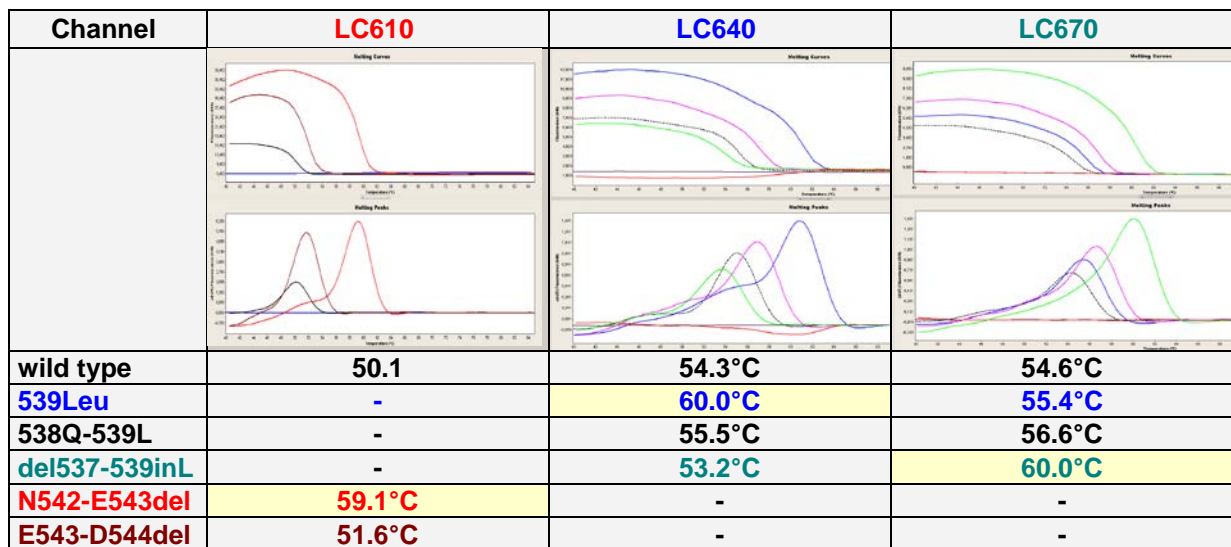


Fig.1. Sample data for the *JAK2*. Data from LightCycler® 2.0 Instrument. Results obtained from cloned genomic targets.

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

## 11. Interpretation of Data

Samples containing leucemic cells with variations in the regions 537 to 544 can constitute a mixture of wild type and one - or sometimes several - variants. The sensor probes are specific for the indicated mutations; any melting temperature higher than expected for wild type is an evident sign for presence of the respective variant. The left part of the melting curve may be different-shaped or may contain shoulders, depending on the amount of wild type sequence in the sample.

Positive samples with a melting profile different from wild type - in particular those exhibiting any higher temperatures than the wild type - contain probably any other mutations and should be further analyzed by DNA sequencing.

## 12. Version History Red notes mark events require changed procedures, blue mod. sequences

V080327	Release version
V091209	Editorial changes
V121212	<span style="color: red;">Control DNA for mutation E543-D544del included</span>
V130813	Editorial changes, MSDS included
V131022	Wrong colour correlation in: 2. Description corrected
V171111	Patent disclaimer, Stability, Contents

Roche SAP order n° 05879507001



### Notice to Purchaser

These reagents were developed and manufactured by TIB MOLBIOL GmbH, Berlin, Germany ([www.tib-molbiol.com](http://www.tib-molbiol.com))  
 Customs Tarif no.(HS code) 2833 0000

Weight: 42 gram