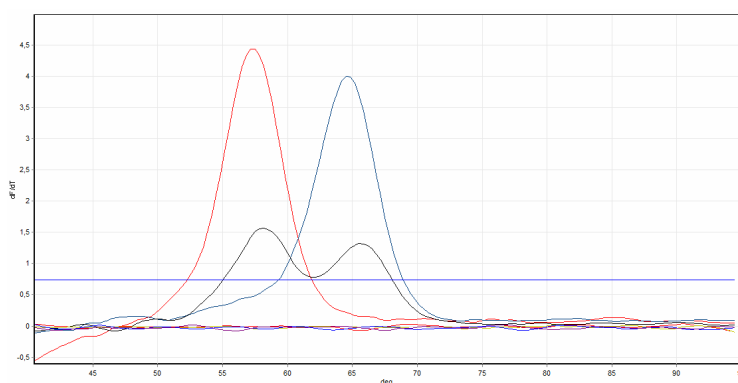




Factor II 20210 RG

Real Time PCR system for the detection of the G20210A mutation in the human coagulation Factor II gene




Reg. MSP pending

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 ATGen SISTEMAS MOLECULARES	Code:	IDK-FII		1
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Only for research.

The purchase of this product does not provide a license to carry out patented applications.

Kit Utility

The kit analyzes the presence of a polymorphism which consists in the substitution of a guanine for an adenine in the 20210 (G20210A) nucleotide located in the 3'UT region of the human coagulation Factor II gene.

Principle of the Assay

The analysis for the detection of the Factor II mutation includes a Real Time PCR amplification of the genes' 3'UT segment containing the polymorphic site followed by denature curves of the amplified product.


Introduction: Factor II

The human Prothrombin (Factor II) gene is located in the region 11p11.2 of chromosome 11 and contains 622 aminoacids. The FII G20210A mutation generates an accumulation of RNAm which increases the protein synthesis explaining the pathogenesis of thrombophilia.



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Kit presentation

Color that identifies the kit: Red

The ATGen kit for the detection of the G20210A mutation includes:

- 1 FII 20210 Reaction Mix tube (yellow cap).
- 1 FII Negative Control tube, C- (transparent cap).
- 1 FII tube with positive control DNA (red cap).

All the reagents included in the Real Time FII kit are in liquid solution. The kits are available in two sizes: 20 and 50 reactions.

Necessary materials not provided with the test

- Real Time thermocycler.
- Biohazard disposable container.
- Gloves and robe.
- Suitable pipettes.
- Filtered pipette tips.
- DNA y DNase PCR free tubes.
- Vortex.

Precautions

1. Only for *in Vitro* use.
2. All samples, reagents and controls should be considered as potentially infectious.
3. Do not use this kit after the expiration date indicated on the package.



Storage and Stability

The kit must be stored at -20°C in order to assure its optimal performance through the expiration date indicated on the package. The kit allows up to 6 defrosting cycles. In case additional defrosting would be needed, we recommend to aliquot the reaction mix after the first defrosting.

Specimen Characteristics


The specimen is a DNA solution with a 150-200 ng/μl concentration, apt for PCR amplification.

ATGen recommends obtaining the DNA from blood specimens by using its ADN Facil kit.



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Protocol

Pre-amplification area:

If possible, perform all manipulation in cold room.

Preparation of the amplification mix:

1. Preparation of the PCR mix:

- 1.1. Defrost the reaction mix, avoiding direct light exposure.
- 1.2. Homogenize the reaction mix.
- 1.3. Preparation of the amplification mix :
 - 1.3A. Place the PCR tubes in a tube rack. Use one tube per test sample and 2 additional tubes for positive and negative controls.
 - 1.3B. Add 18 μ L of the reaction mix to each tube and label them.
 - 1.3C. Add 2 μ L of each sample of DNA to the correspondent tube.
 - 1.3D. Add 2 μ L of positive control DNA to the positive control tube.
 - 1.3E. Add 2 μ L of negative control to the negative control tube.

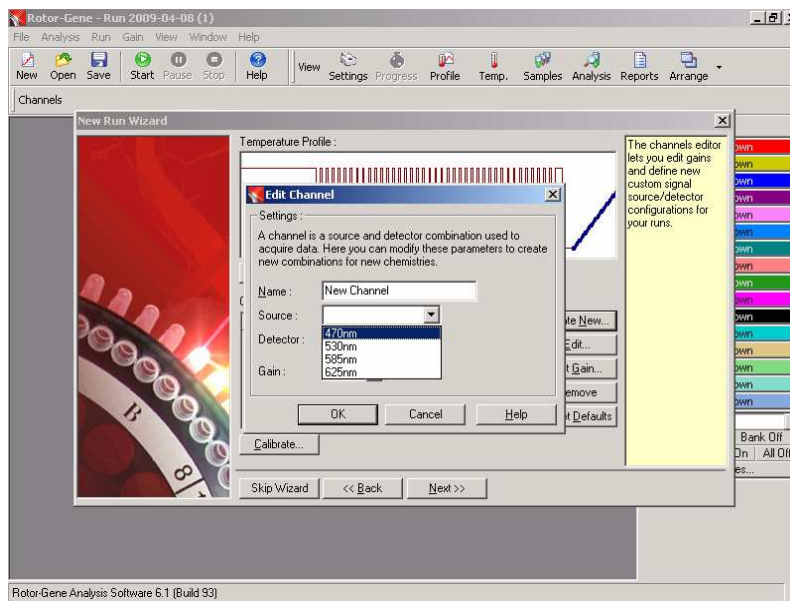
2. Programming the instrument:

- 2.1. Place the tubes in the Rotor-Gene 3000 Real Time thermocycler.

NOTE: In case it is the first time you set the thermocycler, read the following instructions (if not go to 2.2):

- 2.1A. Open the application and select the option **Empty Run**. Then select **New**. In the next window, select the type of tube you are using and then click **Next**.
- 2.1B. In the new window select 20 μ l as reaction volume and then click **Next**.
- 2.1C. In the **New Run Wizard** window, select **Create New** as shown:

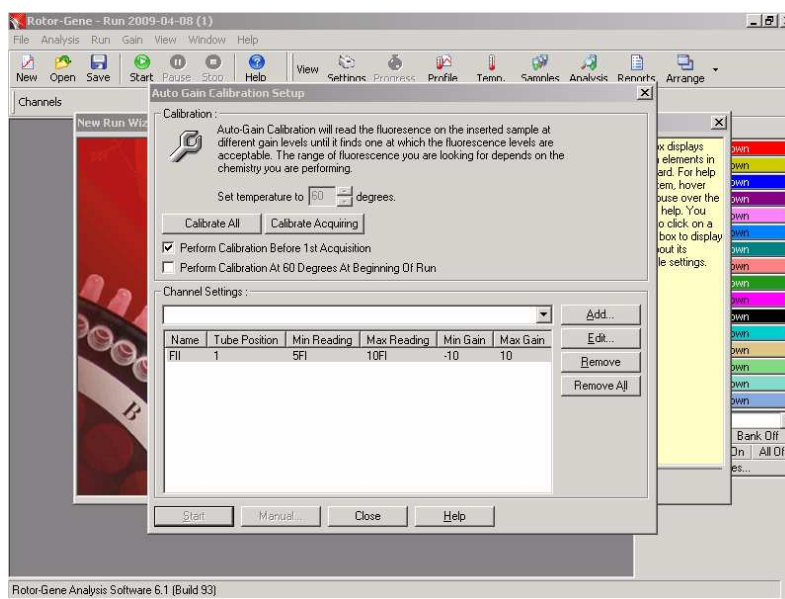




Then complete the chart as follows and then click **ok**.

Name	FII
Source	470 nm
Detector	660 hp

In the window **New Run Wizard** click **calibrate**. In the new window go to Channel Setting and select **channel FII**, and then click **Add**. Indicate **Tube position 1**, **Min reading 5**, **Max reading 10** and then **ok**. Then select the option **Perform calibrate before 1ST acquisition** as shown in the following figure. Then click **Close**.



2.1D. Select **Edit Profile** and fill the cycling data as follows:

	Temp. C	Time	Acquisition
Hold	95	3 min.	No
40 cycles	95	15 sec	No
	56	20 sec	Channel FII
	72	10 sec	No
1 cycle	95	15 sec	No
	40	60 sec	No
Melt	40 a 85	1°C/s	Channel FII

Then click **OK**

2.1E. In the next window select **Save Template** using the name **FII Real Time**.

2.1F. Select **Start Run** and go to step 2.7.

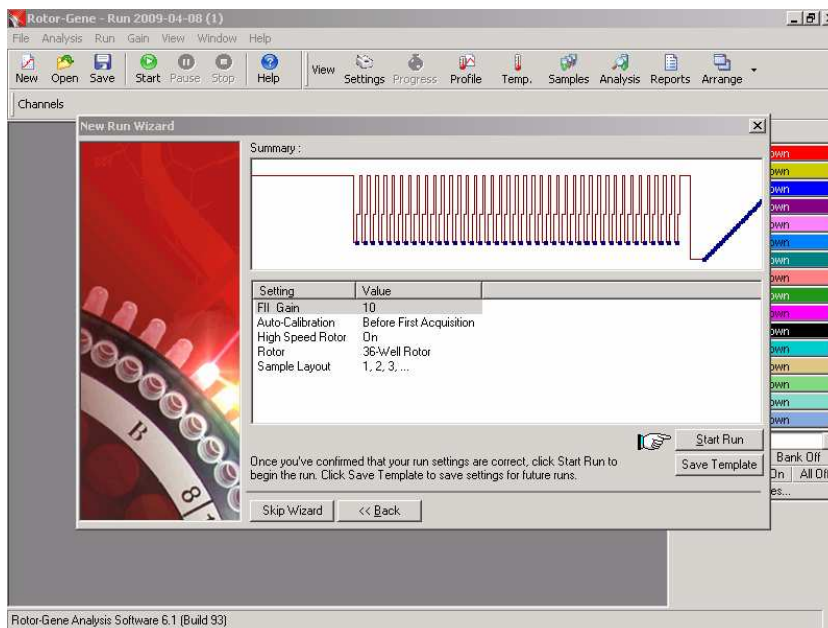
2.2. Initiate the application Rotor Gene in the **New Run** window. Select **FII Real time** and then **New**.

2.3. Select the 36 position rotor and then the type of tubes that are being used, as shown in the figure. Then click **Next**.

2.4. Select 20 µl as reaction volume. Then click **Next**.

2.5. The application will show the following window:





In case you want to verify the cycling program go to step 2.1, if not click **Start run**.

2.6. Save the file with a chosen name. Then click **Accept**.

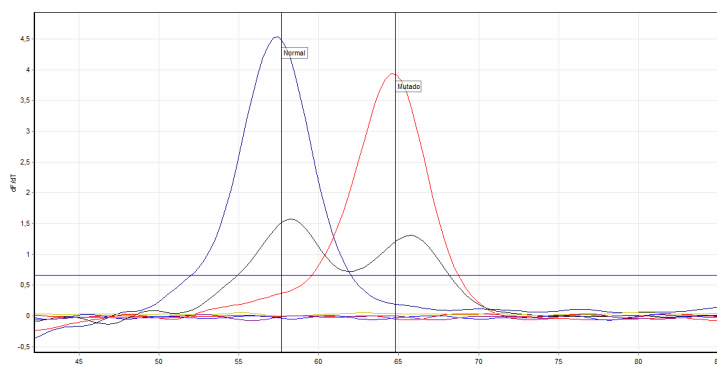
2.7. Introduce the data corresponding to each sample. To close the window click **Finish**.

3. Obtention of test results:

3.1. Once the run finishes, select **Analysis**. In the next screen select **melt** and then **Melt.FII Real Time**. Click **Show**.

3.2. The following graphic will be shown:





Blue line: Shows a normal homocygote (56 °C peak).

Red Line: Shows a mutated homocygote (65 °C peak).

Black line: Shows an heterocygote (56°C peak and 65°C peak).

- 3.3.** In the Peak bin section (on the right part of the screen) select **New**.
- 3.4.** Select the lower temperature peak; when the **Edit Bin**, window opens, complete the chart as shown:

Name	Normal
Position	56
Width (Degrees)	0,5

Then click **Ok**

- 3.5.** Repeat from step 3.2 to 3.3, with the higher temperature peak and the following data:

Name	Mutated
Position	65
Width (Degrees)	0,5

- 3.6.** Select **Genotypes** and complete the chart as follows:

Normal Homocygote	NN	Bin A	-
Heterocygote	MM	Bin A	Bin B
Mutated Homocygote	NM	-	Bin B

- 3.7.** In order to validate the assay it is necessary to verify the presence of the 56°C and 65°C peaks, corresponding to the heterozygote positive control. Regarding the negative control, no signal should be observed over the threshold. If these conditions are not verified, it is not possible to validate the assay and inform the test results.

